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The Journal of Laboratory and Clinical Medicine

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ORIGINAL ARTICLES

CALCIUM SULPHID, AS THE CHEMICAL AND CLINICAL ANTIDOTE FOR MERCURIC CHLORID POISONING, WITH EXPERIMENTS AND CASE REPORTS*

BY J. H. WILMS, M.D., CINCINNATI, OHIO.

AN effective antidote should render a poison innocuous. A chemical compound has an antidotal action when its elements are loosely enough combined, that upon coming in contact with another chemical compound in the human body, their elements have so great an affinity that new compounds are formed which are inert. Once this union takes place the tissues are relieved of the destructive elements of the poison, and regenerate and resume their physiological function. It has often been claimed that an organ, once damaged, cannot regenerate. This, however, has been disproved by the following experiments:

My first experiment demonstrated to me that a clinical as well as chemical antidote has been found in calcium sulphid. This has been verified in repeated experiments by myself and others.

A hypothetical explanation may be found in the following simple, approximately accurate equation:

$$CaS + HgCl_2 = CaCl_2 + HgS.$$

Mercuric sulphid (HgS) and calcium chloride ($CaCl_2$) thus formed are two almost inert chemical compounds. This is the test tube chemical reaction and forms the hypothesis of my animal experimentation.

THE METHOD OF POISONING THE ANIMAL WITH BICHLORID OF MERCURY.

A compressed tablet containing mercuric chlorid, 7.3 gr. and ammonium chlorid 7.7 gr., which I thought was the most insoluble tablet on the market, was used. It was so hard that in a half hour it would not begin to dissolve

*From The B. Merrill Ricketts Experimental and Surgical Research Laboratory, Cincinnati, Ohio.

in a half pint of water without agitation. Some tablets dissolve in a half minute in the same amount of water, and dissolve in less time in a one per cent hydrochloric acid solution.

The tablet was thrown into the dog's throat, the mouth of the animal being held wide open. At times the tablet would be swallowed whole, and sometimes chewed and then swallowed. The variations in solubility in the stomachs of the different dogs were as follows:

ANIMAL.	AMOUNT OF TABL. VOMITED.	TIME INTERVENING BETWEEN TAKING AND VOMITING PART OF TABLET.
Dog No. 1	1/3 tablet, (2 gr. $HgCl_2$)	5 minutes
Dog No. 2	.1 tablet, (.75 gr. $HgCl_2$)	16 "
Dog No. 3	None (but profuse vomiting)	4 "
Dog No. 4	.1 tablet (.75 gr. $HgCl_2$)	3.5 "
Dog No. 5	1/3 tablet (2 gr. $HgCl_2$)	3 "
Dog No. 6	None (but profuse vomiting)	2.5 "
Dog No. 7	1/3 tablet (2 gr. $HgCl_2$)	4 "

Average solubility 6 gr. $HgCl_2$ in 5 to 6 minutes.

This table will show the unusual solubility in the dogs' stomach of the very insoluble tablets.

In the experiments which follow, the dogs received no antidote. The experiments show the extensive destructive action of bichlorid of mercury in dogs.

EXPERIMENT NO. 1.

Nov. 2, 1915.—White bull terrier, weight 16 lbs. (No antidote).

One tablet of bichlorid of mercury (7.3 grains) and ammonium chlorid (7.7 gr.) was thrown down the dog's throat. Vomiting commenced in a few minutes; severe intestinal tenesmus and strangury followed.

Nov. 3, 1915.—Dog was listless.

Nov. 4, 1915.—Dog listless and lay in a cramped position.

Nov. 6, 1915.—Dog very sick.

Nov. 7, 1915.—Dog found dead.

Postmortem.—Intestines were red and inflamed; stomach contained a thick coffee-colored fluid, a round ulcer about one centimeter in diameter was found on the anterior surface near the pylorus; submucous clot-like masses were found about the pylorus, varying in size from two to five millimeters in diameter. The duodenum was red and inflamed; bile, thick and gritty; the kidney cortex was red and streaked; the medulla nephrica was congested, proportion cortex one to medulla three; red fluid in the calices. *Chest.*—Both lungs hepatized. *Heart.*—Ulcer on the visceral layer of the pericardium over the terminals of the coronary vessels; pericardium thickened. Myocardium inflamed; musculi pectinati markedly inflamed; subendocardial ecchymoses in both ventricles; esophagus inflamed.

EXPERIMENT NO. 2.

May 12, 1916.—Small white dog, 15 lbs. (No antidote).

4:35 P. M. A 7.3 gr. bichlorid of mercury tablet thrown down dog's throat.

4:37 P. M. Slight blood-stained vomiting was followed by a frequent vomiting of thick, yellow, glary mucus.

May 15, 1916.—Dog found dead in the afternoon. Postmortem findings similar to those of Experiment No. 1. (Specimens were used for chemical analysis.)

EXPERIMENT NO. 3.

May 16, 1916.—Yellow setter, 40 lbs. (No antidote).

4:56 P. M. A 7.3 gr. tablet bichlorid of mercury thrown down throat.

4:56.75 P. M. Dog restless.

4:57.5 P. M. Dog licks chops.

4:58.5 P. M. Nausea.

4:59 P. M. Vomiting and violent straining; vomitus cruentus; stringy and tenacious mucus vomited in large quantities.

5 P. M. Vomited 1/3 of tablet.

5:01 P. M. Readministered the piece of tablet.

5:02 P. M. Vertigo; very sick.

May 20, 1916.—Dog killed for specimens.

Postmortem revealed similar condition to first experiment with the exception that the stomach was very much corroded. (Specimens used for chemical analysis.)

EXPERIMENT NO. 4.

This experiment will show that enough mercury is vomited and excreted to poison another dog.

A medium-sized dog was placed in the cage which had been occupied by the dog in Experiment No. 7. The dog died six days after being placed in the cage, after showing symptoms of mercury poisoning: i.e., vomiting and diarrhea.

Postmortem.—Pregnant bitch; lungs, hypostatic pneumonia; duodenum, inflamed and extensive ecchymoses of the submucosa. Stomach inflamed and ecchymosed submucosa. Bile dark. Kidneys swollen, dark red, capsule peels off, edges pout when cut. Cecum and ascending colon contain many pin-head ulcers. Heart contained a fatty-looking thrombus that was attached to the walls of both ventricles.

After this experiment we saw that it was necessary to antidote the cages and all the other animals, in the laboratory, with calcium sulphid. This accounted for the death of several dogs that had died of chronic mercurialism.

The following experiments will show the effect of the antidote, calcium sulphid, on the dogs that received bichlorid of mercury.

EXPERIMENT NO. 5.

Oct. 16, 1915.—A good sized dog, about 30 lbs. in weight, with a brown woolly coat. 4 P. M. one tablet containing 7.3 gr. of bichlorid of mercury and 7.7 gr. chlorid of ammonia was given per mouth. In a few minutes the dog vomited. This was followed by frequent vomiting and rectal tenesmus.

Oct. 18, 1915.—Dog thin and sick; bloody diarrhea. Forty-eight hours after receiving bichlorid of mercury, with no antidote in the interim, a solution of calcium sulphid which had been boiled and filtered through cotton (7.5 grains CaS to 7.5 ounces of tap water) was administered intravenously.

Oct. 20, 1915.—Dog much improved.

Oct. 30, 1915.—Dog well and lively.

Nov. 4, 1915.—Dog, playful, looks clean and has a healthy glossy coat.

Nov. 8, 1915.—Dog very lively.

Nov. 17, 1915.—Unusually lively.

Nov. 29, 1915.—Reported the experiment to the Cincinnati Academy of Medicine and presented the dog.

Dec. 2, 1915.—Killed the dog by bleeding.

Postmortem.—Dog well nourished and fat. Tissues healthy in appearance. Kidneys clear and normal microscopically. Liver paler than normal (due, perhaps, to the hemorrhage). Bile thin and amber-colored. Stomach and intestines normal.

EXPERIMENT NO. 6.

March 31, 1916.—White dog, mongrel, weight 19 lbs.

4:13.5 P. M. 7.3 grs. bichlorid of mercury tablet per mouth.

4:15 P. M. Restless.

4:16 P. M. Straining, urinated profusely; dark colored urine.

4:17 P. M. Vomiting thick tenacious mucus.

4:18.5 P. M. Violent retching; vomitus bile-stained.

4:27 P. M. Vomitus cruentus.

4:28 P. M. Bowels moved.

4:29 P. M. Straining to stool; rectal tenesmus; a small piece of tablet vomited, which was readministered.

April 1, 1916.—Dog very sick and listless.

April 2, 1916.—10 A. M. So very sick, dog was expected to die.

4 P. M. Antidote, a solution of calcium sulphid, 7.5 gr. to 7.5 ounces of water, the solution boiled and filtered through cotton, was administered intravenously. About an ounce of the solution was lost during the operation. While injecting the antidote the dog made several gasps.

April 4, 1916.—Dog much improved.

April 6, 1916.—Dog apparently normal.

April 28, 1916.—Dog normal and lively.

June 6, 1916.—Dog killed.

Postmortem.—All organs macroscopically normal. Mr. R. A. White, curator of the laboratory, and several pathologists examined, microscopically, specimens of the kidneys and reported normal kidneys.

EXPERIMENT NO. 7.

April 8, 1916.—Black water Spaniel.

3:53.5 P. M. One tablet bichlorid of mercury (7.3 grains) administered by mouth.

3:57 P. M. Vomited.

3:58 P. M. Blood-stained vomitus.

4:08 P. M. Rectal tenesmus and stool.

April 9, 1916.—Dog very sick.

April 10, 1916.—Dog lies quietly.

April 11, 1916.—4 P. M. 72 hours after receiving the bichlorid of mercury, the antidote was administered intravenously (a solution of calcium sulphid, 7.5 grains in 7.5 ounces of water, boiled and filtered through cotton).

April 14, 1916.—Dog died some time during the night.

Postmortem.—Lungs, double pneumonia. Pericardium thickened. Heart contained large fatty-looking thrombus, attached to the right ventricular wall. The left ventricle showed subendocardial and myocardial ecchymoses. The stomach mucous membrane was ulcerated, involving the whole lesser curvature. Esophagus pale. Small intestine had many ulcers; large intestines had ecchymoses and bloody stool. Kidney, cortex yellow, left kidney markedly congested; liver congested, (venous). Bile, dark and thick.

EXPERIMENT NO. 8.

April 20, 1916.—Hound.

3:44 P. M. 7.3 grains of bichlorid of mercury tablet administered by mouth.

3:47.5 P. M.—Vomiting; a small piece of tablet vomited which was readministered per os. This was followed by frequent vomiting of tenacious and glary mucus.

3:53 P. M.—Rectal tenesmus.

3:54 P. M. Strangury and rectal tenesmus.

April 22, 1916.—4 P. M. Solution of 7.5 gr. calcium sulphid in 7.5 oz. water was administered intravenously, into the external jugular vein.

April 23, 1916.—Dog not so well as others.

April 24, 1916.—No improvement.

April 25, 1916.—Dog looks very sick.

April 26, 1916.—Bloody stool.

April 28, 1916.—Stool bloody.

April 30, 1916.—Dog died during night.

Postmortem.—Dog emaciated, bloody stool. Stomach dilated and inflamed; contained a brownish-green fluid; inflammation and ecchymoses of the rugæ. Duodenum inflamed. Gall-bladder thickened and bile thick. Lungs somewhat congested. Heart contained a fatty-looking thrombus attached to the walls of the right ventricle.

Later is was discovered that the calcium sulphid used in this and experiment No. 7 was an old stock solution which had been in the laboratory for some time, and had lost most of its sulphur. Since sulphur is the active agent in the antidote, the solution was of very little value. This was borne out by a number of experiments, in which the same solution was used.

Wilhelm Ostwald gives the following explanation showing the chemical change in the old solution. "The salt . . . . is only very slightly soluble

in water, but is decomposed on being warmed for some time with it, calcium hydrosulphide passing into solution and calcium hydroxide remaining behind: $2\ CaS + 2\ H_2O = Ca(HS)_2 + Ca(OH)_2$."

EXPERIMENT NO. 9.

May 12, 1916.—Large dog, setter.

4:25 P. M. A 7.3 grains tablet bichlorid of mercury given per mouth.

4:28 P. M. Vomiting. 1/3 of tablet found in vomitus. This piece was readministered. This was followed by frequent vomiting.

4:42 P. M. Stool. Rectal tenesmus.

4:47 P. M. Blood streaked vomitus.

May 14, 1916.—4:25 P. M. Antidote, 7.5 grains calcium sulphid in 7.5 oz. water, after being boiled and filtered, injected intravenously.

May 16, 1916.—Dog lively.

May 18, 1916.—Dog apparently well.

May 24, 1916.—Steady gain in weight.

June 6, 1916.—Dog killed.

Postmortem.—Dog well nourished and fat. Organs appeared to be normal. Kidneys examined microscopically by Mr. R. A. White and pronounced normal.

This experiment was done to test the stability of the oily suspension of calcium sulphid, and it proved to be as unstable as the watery solution. The same preparation was used 2 weeks later, and the dog it was used on died of clonic convulsions in about two hours and a half after it received the injection.

EXPERIMENT NO. 10.

July 17, 1916.—9:05 A. M. 7.5 grains of $HgCl_2$ administered.

3:45 P. M. Calcium sulphid given intravenously, 7.5 grains to one ounce of sweet almond oil.

The dog steadily improved.

Aug. 10, 1916.—The dog is fat and healthy.

SUMMARY OF THESE EXPERIMENTS

Dog of Ex. 5, 48 hour antidote, recovered.

Dog of Ex. 6, 48 hour antidote, recovered.

Dog of Ex. 7, 72 hour antidote (old calcium sulphid solution), death on 6th day.

Dog of Ex. 8, 48 hour antidote (old calcium sulphid solution), death on 10th day.

Dog of Ex. 9, 48 hour antidote, recovered.

Dog of Ex. 10, 6 hour antidote (CaS in oil). Recovered.

These experiments showed to me so clearly the value of calcium sulphid as an antidote for mercuric poisoning that demonstrations were discontinued.

The object of the following experiment is to show the effect of mercuric sulphid, when administered by mouth.

EXPERIMENT NO. 11.

May 8, 1916.—3:30 P. M. A solution of 7.5 grains of bichlorid of mercury was mixed with 7.5 grains of calcium sulphid in a test tube and the resulting solution was thrown down a dog's throat. No symptoms developed, with the exception of a slight vertigo, which may not have been the result of this solution.

May 24, 1916.—Dog very well and lively, having shown no symptoms at any time.

May 31, 1916.—Dog used for other experimental purposes. No symptoms were present.

The following experiment was performed to determine the immediate effect of introducing a calcium sulphid solution intravenously.

EXPERIMENT NO. 12.

Oct. 12, 1915.—A rabbit received intravenously, one grain of calcium sulphid to one ounce of water, and showed no immediate symptoms.

Oct. 16, 1915.—Rabbit normal

Oct. 30, 1915.—Rabbit normal.

Nov. 9, 1915.—Rabbit died, after receiving a dip.

Postmortem.—Animal thin and emaciated. Abdominal and thoracic cavity negative. It is hardly possible that the calcium sulphid was the cause of the rabbit's death.

The following is a résumé of experiments performed by Mr. Frank Maltaner, M.A. of the laboratory of the Cincinnati Board of Health, to verify my experimental work with calcium sulphid. These experiments were done at the instigation of Dr. J. H. Landis, Health Officer of the city of Cincinnati. Quoting Mr. Maltaner:—

"NOTES ON THE EFFECT OF BICHLORID OF MERCURY ON RABBITS, AND THE VALUE OF THE CALCIUM SULPHID ANTIDOTE USED ON THESE ANIMALS.

"*Administration of the Poison.*—The chemically pure bichlorid of mercury was filled into gelatin capsules and forced down the animal's throat. After taking a lethal dose of the poison, the animals refused to eat or drink. Profuse defecation usually occurs, followed after about two hours by more or less diarrhea. There is continued refusal of food and diarrhea and gradual loss of weight, followed by death in from several hours to four days.

"Examination of the cadaver shows usually some fluid in the peritoneal cavity (not constant). The lungs sometimes show acute inflammatory areas. The veins of the peritoneum, stomach, mesentery, and heart are injected. The small intestine has a greyish, metallic color and putty-like consistency. The kidneys show more or less swelling, and are greyish white in color and embedded in an extensive fatty capsule. The spleen is small and metallic. The stomach is large and tense, and the inner coating is much inflamed and corroded.

"Mercury was detected in the heart, liver, spleen, kidney, blood, and urine. Mercury was also detected in the blood drawn from the marginal vein of the ear, 15 minutes after the administration of the bichlorid by mouth.

"The lethal dose of the bichlorid, when administered by mouth, proved to be .25 grain per pound of animal. This dose invariably killed in from 12 hours to 4 days. A dose of .2 grain per pound or less had no apparent effect upon the rabbits.

"*Administration of the Antidote.*—The antidote is an aqueous solution of calcium sulphid in a dilution of one grain to the ounce of distilled water. It is prepared by dissolving the calcium sulphid in boiling water and filtering hot. It is then ready for use. We used both the product prepared for Dr. Wilms by the Merrill Chemical Co. and some prepared ourselves with equal success.

"The dosage adhered to was the same as recommended by Dr. Wilms: viz., one grain of the antidote to each grain of bichlorid taken. It was given intravenously, the marginal vein of the ear being selected in each case.

"Two rabbits that received .25 grain per pound weight of bichlorid were given the antidote after 2.25 hours and 5.75 hours respectively and recovered completely.

"One rabbit received .5 grain per pound weight of bichlorid. The antidote was given after 2.75 hours with complete recovery of the animal.

"One rabbit received .25 grain per pound weight of bichlorid and was given the antidote 5.5 hours later. This rabbit died after 3 days showing typical bichlorid poisoning symptoms and pathology.

"CONCLUSIONS.—The calcium sulphid antidote is certainly of value in the treatment of rabbits poisoned with bichlorid of mercury.

"The antidote must be given, if possible, in less than five hours after the poison has been administered in rabbits.

"The poison is very rapidly absorbed, appearing in the blood (taken from the ear) in less than 15 minutes.

"REMARKS.—The value of the antidote probably lies in the formation of the relatively insoluble, double salt mercuric sulphid mercuric chlorid (HgS. $HgCl_2$) and the very insoluble mercuric sulphid.

"Both of these substances are formed in the test tubes, the formation of the insoluble sulphid depending upon the excess of calcium sulphid used."

The following are Mr. Maltaner's Experiments:

RABBIT NO. 1.

Rabbit of 2 pounds weight; received .5 grain of mercury bichlorid at 10:40 A. M. April 24.

2 P. M. Diarrhea.

4 P. M. Received .5 gr. of calcium sulphid intravenously.

April 26, A. M. 1-2/3 pounds weight.

April 27, A. M. Dead.

Postmortem.—Serous fluid in peritoneal cavity; veins of stomach, heart, mesentery, and peritoneum injected; small intestine has greyish metallic appearance and putty-like consistency; lungs inflamed; kidneys greyish white swellings; spleen small and metallic; stomach tense and inner coating much inflamed and corroded.

RABBIT NO. 2.

Rabbit of 2 2/3 lbs. weight received .67 grain of mercury bichlorid at 11 A. M. on April 24.

2 P. M. Diarrhea.

April 25, A. M. Found dead

Postmortem appearance similar in every way to first rabbit.

RABBIT NO. 3.

Rabbit of 2 lbs. weight received .5 grain of mercury bichlorid at 9:26 A. M. April 27.

11:45 A. M. Received .5 grain solution of calcium sulphid intravenously.

1:45 P. M. Had slight diarrhea.

April 28. A. M. In good condition and weighed 1 2/3 lbs.

May 5. Complete recovery; weight 2 lbs.

May 18. Weight 2 1/3 lbs.

RABBIT NO. 4.

Rabbit of 3 lbs. weight received .75 grain of bichlorid of mercury at 9:40 A. M. April 27.

3:25 P. M. Received .75 grain of calcium sulphid solution intravenously.

April 28, 8 A. M. Slight diarrhea; slight loss in weight.

April 29. Regained weight; good condition.

May 18. Good condition; weight 3 lbs.

RABBIT NO. 5.

Rabbit of 4 lbs. weight received 2 grains of bichlorid of mercury at 9 A. M., May 3.

11:45 A. M. Received 2 grains of calcium sulphid solution intravenously.

2 P. M. Diarrhea, profuse and bloody.

May 4, 8 A. M. No diarrhea; weight 3 2/3 lbs.

May 12. Good condition; normal weight.
June 2. Good condition; normal weight.

RABBIT NO. 6.

Rabbit of 4 lbs. weight given 1 grain of bichlorid of mercury at 9:35 A. M., May 8.
10:30 A. M. Diarrhea.
May 9, A. M. Dead.
Postmortem condition similar to rabbit No. 1; heart, liver, spleen and kidneys gave chemical test for mercury.

RABBIT NO. 7.

Rabbit of 3 lbs. weight received 1.5 grains of bichlorid of mercury 10 A. M., May 9.
May 10, A. M. Dead.
Postmortem same as rabbit No. 1; mercury was detected in the blood taken from ear vein 15 minutes, 30 minutes, 45 minutes after the bichlorid was given by mouth; the heart, liver, spleen, kidney, blood and urine gave chemical tests for mercury.

RABBIT NO. 8.

Rabbit of 3 lbs. weight given .15 grain of bichlorid of mercury at 3:05 P. M., May 15.
May 26. No reaction; weighs 3.5 lbs.

RABBIT NO. 9.

Rabbit of 3 lbs. weight given .3 grain of bichlorid of mercury at 3:05 P. M., May 15.
May 26. No reaction; weighs 3.5 lbs.

RABBIT NO. 10.

Rabbit of 3 lbs. weight given .45 grains of bichlorid of mercury on May 18.
June 2. No reaction; gain in weight.

RABBIT NO. 11.

Rabbit of 3 lbs. weight given .6 grain of bichlorid of mercury on May 18.
June 2. No reaction; gain in weight.

RABBIT NO. 12.

Rabbit of 3 lbs. weight given .75 grain of bichlorid of mercury on May 26.
May 27. Lost .5 lb. in weight.
May 30. Dead.
Typical mercury poisoning.

RABBIT NO. 13.

Rabbit of 3 lbs. weight received .9 grain of bichlorid of mercury on May 26.
May 27. Lost .5 lb. in weight.
May 29. Dead.
Typical mercury poisoning.

CASE REPORTS.

In June, 1915, through the courtesy of Dr. Oscar Berghausen, I was called to the Cincinnati General Hospital to treat a case of bichlorid of mercury poisoning. Upon my arrival at the institution at 1 P. M. I found a female patient had taken 110 grains of bichlorid of mercury the evening before with suicidal intent. She was about 30 years of age, and in a moribund condition. After the exhibition of five grains of calcium sulphate by mouth every half hour for three or four hours, she died. This case suggested to me that if I could have given her a solution of calcium sulphid intravenously I might have been able to neutralize the bichlorid of mercury, and that the neutralization would have been rapid enough to have saved her.

On April 19, 1916, I was consulted by Mr. S. of Los Angeles, Cal., whose wife had used by mistake 30 hours previously a 7.5 gr. of bichlorid of mercury tablet intravaginally, thinking it was an antiseptic wafer. The antidote formula, 1 grain of calcium sulphid to 1 ounce of water was telegraphed to her physician, Dr. C. W. Decker.

The patient was given intravenously 7.5 grains of calcium sulphid dis-

solved in 7.5 ounces of water, the solution having been boiled and filtered through cotton. After several days she began to improve and made an uneventful recovery.

On May 25, 1916, Dr. L. N. Denman informed me that he had a patient who had used a 7.5 grain bichlorid of mercury tablet intravaginally to prevent pregnancy. He saw her a short time after the tablet had been introduced, and made a careful examination of the vagina for the tablet, but was unable to find it. Her vagina and vulva were swollen and inflamed. He prescribed one grain of calcium sulphid per mouth every two hours and recommended a douche, 1 dram of calcium sulphid to the ounce of water per vagina. He saw this woman twice after taking the bichlorid tablet and then not again till July 16, 1916. She had been perfectly well in the meantime and had gone away on a trip.

The report of these two cases should be compared with three cases reported by Dr. G. B. Schildecker, American Journal of Obstetrics, 1911, lxiii, 473-475.

Case I.—A physician made a careful examination 35 minutes after a bichlorid of mercury tablet had been introduced by a woman into her vagina to prevent pregnancy, and he could find no trace of the tablet. Patient died in four days. The postmortem showed parenchymatous degeneration of the liver; marked fatty degeneration of both kidneys; marked fatty degeneration was noticed along the nutrient vessels of the heart. Treatment was not mentioned.

Case II.—One tablet of bichlorid of mercury was inserted into the vagina to prevent pregnancy. Treatment was enteroclysis. The patient lived two weeks, died of perforation of the colon with peritonitis. Autopsy showed similar condition to Case I.

Case III.—One tablet of bichlorid of mercury was introduced into the vagina to prevent pregnancy. Treatment, vagina was washed out with hot water in twenty minutes, followed by enteroclysis of saline solution. Patient died on the seventh day. Autopsy showed same results as the other cases and passive hemorrhage in the most dependent portions of the peritoneal cavity. A large amount of bloody serous exudate beneath the brain and membranes.

His conclusions were that the tablet was absorbed in twenty minutes, and that the tablet should be removed in a few minutes. The person suffering from such an accident has a small chance of recovery because of the far-reaching effects of the poison, as shown by the autopsy findings in these cases.

On April 1, 1916, through the courtesy of Dr. J. H. Landis of the staff of St. Mary's Hospital, Cincinnati, Ohio, I was permitted to use calcium sulphid intravenously on a man, 39 yrs. of age, who had taken 7.5 grains of bichlorid of mercury the preceding night about 1 A. M. The following day when I saw the man at 3:30 P. M., he had a bloody diarrhea and suppression of urine, accompanied by a great deal of pain in the abdomen, and a very sore mouth. He informed me that he had vomited five minutes after taking the tablet. I gave him intravenously, a freshly prepared solution of calcium sulphid which had been boiled and filtered through cotton, 7.5 grains to 7.5 ounces of water. The next day he was so much improved that his bowel symptoms had prac-

tically disappeared. He had had no stool after the injection, and was passing a normal amount of urine. The second day after the injection he was suffering from a pyorrhea alveolaris and stomatitis, for which I prescribed a grain of calcium sulphid per mouth, every hour. He made an excellent recovery and I presented him to the Cincinnati Academy of Medicine ten days after he had taken the bichlorid of mercury. He informed me that he had gained seven and a half pounds in weight in ten days.

On April 27, 1916, Dr. Hays was called to treat a girl, A. D., 20 yrs. old, who had taken a 7.3 grain bichlorid of mercury tablet by mistake for a headache tablet at 6:30 A. M. Shortly after, she became very sick and vomited. He sent her to St. Mary's Hospital where she received a gastric lavage at 8:30 A. M., after which she was given calcium sulphid, one grain every hour. The second day she went home from the hospital and showed no further symptoms.

On June 2, 1916, a 19 year old girl was supposed to have taken two bichlorid of mercury tablets of 7.3 grains each, with suicidal intent. She became very sick shortly afterward and was found wandering in a sort of delirium. She was taken to St. Mary's Hospital and given one grain of calcium sulphid per mouth every two hours, prescribed by Dr. Hays. The next day she was very sick, but the second day after receiving the calcium sulphid, she was so much improved that she was able to leave the hospital. Her recovery was complete and uneventful.

I was consulted by Dr. F. A. Ireton of Newtonsville, Ohio, on May 21, 1916, concerning Mrs. W., aged 35, who had taken one 7.3 grain bichlorid of mercury tablet. She vomited in about ten minutes. He commenced using calcium sulphid about twelve hours after she had taken the tablet. Tenderness was marked all over the abdomen and she passed pieces of mucous membrane from the rectum. Urinary symptoms were not noted. He reports to me on August 2, that she was dismissed June 1, having made an excellent recovery and that he saw her in his office July 5, when he saw no symptoms of the poisoning.

In January, 1916, Dr. J. Kelley Brammer of Cincinnati, Ohio, administered calcium sulphid by mouth, 7.5 grains, to a woman who had taken a 7.5 grain bichlorid of mercury tablet. August 3, 1916, he reports to me in a personal communication that she made an excellent recovery and showed no untoward effects of the poisoning.

I have information of a number of other recoveries following the use of calcium sulphid in bichlorid of mercury poisoning, but the reports of the consulting physicians were too brief or vague to be herein reported.

I should like to call attention to the possible value of calcium sulphid as an antidote for industrial chronic mercurialism found among felt hat makers, workers in mercury, furriers, dentists, etc. In these cases an occasional dose of calcium sulphid should completely neutralize all the mercury.

SUMMARY AND CONCLUSIONS.

In advanced cases of mercuric chlorid poisoning the intravenous method of injecting calcium sulphid solution grain for grain of the bichlorid of mercury taken, is the safest and most rapid. An advantage of the intravenous method lies in the assurance that the patient receives the required amount of the antidote, and that it is more direct. Calcium sulphid may also be administered by mouth when the intravenous method is not practicable. When the discomfort or the condition of the patient warrants it, both methods may be used. The use of calcium sulphid by mouth may be continued till all symptoms of mercurialism have disappeared, since it is nontoxic. Recovery has taken place, when the antidote was administered by mouth, as late as twelve hours after taking 7.5 grains of bichlorid of mercury, and thirty hours when administered intravenously.

The minimum lethal dose of bichlorid of mercury in the human being is one decigram. (Barbour.)

The calcium sulphid solution must be freshly prepared for intravenous exhibition or it loses its sulphur and becomes ineffective. Calcium sulphid solution should not be used stronger than one grain to the ounce of water. The solution should be boiled and filtered through cotton. It should preferably be given in the median basilic or the median cephalic vein.

Calcium sulphid is the quickest, simplest and surest antidote for mercurial poisoning used at the present time. It has been proved clinically and in the laboratory.

Experiments showed that mercuric chlorid poisoning produced a wide pathologenesis notably, gastritis, ulcer of the stomach, hepatitis, duodinitis, colitis, endocarditis, ulcerative pericarditis, pneumonitis, tubular nephritis (which is manifested by complete suppression of the urine) and a number of other very marked pathological changes, microscopically and macroscopically manifested. All these tissue changes become normal with the exhibition of one dose of calcium sulphid in solution intravenously grain for grain of poison taken.

When given by mouth, it should be administered in the tablet or crude drug form, in two to five grain doses every hour, until an excessive amount is taken. A grain for a grain is four times the amount of calcium sulphid necessary to neutralize mercuric chlorid in the test tube.

The calcium sulphid solution, if deteriorated, will produce severe convulsions owing to the free action of calcium on the spinal cord hence the necessity of a fresh solution. Another danger is the possibility in deteriorated solutions, of hydrogen sulphid which is a fatal poison being present.

Local antidoting in the stomach by the use of whites of eggs and lavage with large quantities of water is useless; bichlorid of mercury is so rapidly absorbed from the stomach that very little remains in the stomach unabsorbed at the end of five minutes. The vomiting, by this time, is so profuse that if any free bichlorid of mercury were still present in the stomach, it would not remain long enough to cause further damage.

CALCIUM SULPHID.*

The product commonly known as calcium sulphid or sulphurated lime is a mixture containing calcium sulphid, calcium sulphate and carbon, and is usually prepared by heating calcium sulphate with charcoal and starch to a red heat. The reaction is

$$CaSO_4 + C_3 = CaS + CO_2 + CO.$$

The CO_2 and CO pass off as gases, leaving calcium sulphid and some unchanged calcium sulphate and carbon.

When calcium sulphid (CaS) is dissolved in water a decomposition takes place according to the equation:

$$2\ CaS + 2\ H_2O = Ca(OH)_2 + Ca(SH)_2$$

the products being calcium hydroxide and calcium sulphydrate.

To determine the composition of solutions of calcium sulphid, three solutions were made as follows:

CALCIUM SULPHID U.S.P. (MERCK).

Solution I.	Solution II.	Solution III.
16 grs.	32 grs.	48 grs.
16 fl. oz.	16 fl. oz.	16 fl. oz.

In each case the calcium sulphid was boiled with the water, the solution cooled and filtered through paper, and placed in tightly stoppered pint bottles.

The following quantitative determinations were then made on each of these solutions:

1. 100 c.c. was titrated with $^N/_{10}$ H_2SO_4 to determine the alkalinity of the solutions. In this connection it should be borne in mind that only the alkalinity of the $Ca(OH)_2$ in solution is indicated by this titration. The $Ca(SH)_2$ is also decomposed by the acid, but for each equivalent of H_2SO_4 added, an equivalent of H_2S is liberated, thus neutralizing the effect of the alkalinity of the $Ca(SH)_2$.

The equation is:

$$Ca(SH)_2 + H_2SO_4 = CaSO_4 + 2\ H_2S.$$

2. 50 c.c was titrated with $^N/_{10}$ iodine solution using starch solution as indicator. This indicates the amount of calcium sulphydrate in solution according to the equation:

$$Ca(SH)_2 + 4\ 1 = CaI_2 + 2\ HI + S_2.$$

Instead of calculating these results to calcium sulphydrate they may be calculated to the original amounts of calcium sulphid dissolved before their decomposition into $Ca(OH)_2$ and $Ca(SH)_2$. See table below.

3. 25 c.c. were treated with a slight excess of ammonium oxalate in order to determine the total calcium (hydroxide, sulphydrate and sulphate). Insoluble calcium oxalate is precipitated. This is collected on a filter, ignited at white heat and weighed as CaO. The reactions involved are:

*I am indebted to Mr. H. W. Jones for this study in the chemistry of calcium sulphid.

$$CaSO_4 \text{ (or other calcium salt)} + (NH_4)_2C_2O_4 = CaC_2O_4 + (NH_4)_2SO_4$$
$$CaC_2O_4 + \text{Heat} = CaO + CO_2 + CO$$

Knowing the amounts of $Ca(OH)_2$ and $Ca(SH)_2$ in solution we may now calculate these in terms of CaO, deduct them from the total CaO, and calculate the balance as $CaSO_4$.

Following are the results obtained by these operations:

Solution	$Ca(OH)_2$	$Ca(SH)_2$	CaS	$CaSO_4$
I	.0467 gm. 0.213 gr.	.0674 gm. 0.308 gr.	.0916 gm. 0.418 gr.	.0777 gm. 0.23 gr.
II	.0636 gm. 0.291 gr.	.0922 gm. 0.421 gr.	0.125 gm. 0.572 gr.	0.103 gm. 0.47 gr.
III	.0644 gm. 0.294 gr.	.0922 gm. 0.421 gr.	0.125 gm. 0.572 gr.	0.131 gm. 0.598 gr.

In grams per 100 c.c., and grains per fl. oz.

These results show that the saturation point lies between 2 and 3 grains of calcium sulphid per fl. oz. In other words above 2 grs. per fl. oz. no more material is dissolved.

These solutions are perfectly clear and bright and show no trace of heavy metals when treated qualitatively.

Theoretically there are five possible sulphides of calcium, but only two of these are well-known: viz., the monosulphide (CaS) and the pentasulphide (CaS). The pentasulphide is obtained by heating milk of lime with sulphur.

$$3\ Ca(OH)_2 + 12\ S = 2\ CaS_5 + CaS_2O_3 + 3\ H_2O.$$

This is what is known as Vlemingh's solution.

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A PRELIMINARY MEDICAL STUDY OF THE POSITIVE CASES FOUND IN THE FIRST YEAR'S WORK OF THE MICHIGAN TUBERCULOSIS SURVEY

By Victor C. Vaughan, Jr., M.D., Detroit, Mich.

THE nature and the methods of this survey have already been given in this Journal (Vol. 1, p. 134; Vol. 2, p. 206). Out of 11,528 persons examined, 2957 were diagnosed as positively tuberculous. These cases have been tabulated as shown in Table I, which represents the positive cases found in one of the smaller counties.

TABLE I

Alger Co.

NO.	AGE	SEX	PNEUMONIA	PLEURISY	HEMORRHAGE	LOSS OF WEIGHT	ASSOCIATION		TEMP. PULSE			COUGH	EXPECTORATION	NIGHT-SWEATS	STAGE			LOCATION		RALES	CALMETTE
							MALE	FEMALE	A. M.	P. M.	PULSE				INCIPIENT	MOD.	ADV.	RT.	LEFT		
1	43	M	−	−	+	+	−	−		−	−	+	+	−		+		U	U	−	+
2	21	M	−	−	−	−	−	−		+	−	−	−	−	+			U		+	
3	54	F	−	−	+	−	−	−	−		+	+	+	+	+				L	+	
4	39	M	−	−	−	−	B.	M³S	+		−	−	−	+		+		U	U	−	+
5	50	M	−	−	−	+	−	−	+		−	−	−	+		+		UM		+	+
6	25	F	−	−	−	+	−	−	+		+	−	−	−	+			U	U	−	−
7	25	M	+	−	−	−	−	−	−		−	+	+	−		+		U	U	−	A−
8	32	M	+	−	−	−	−	−		+	−	+	+	−		+		UM	U	−	A−
9	41	F	−	−	−	+	−	−		+	+	−	−	−		+		UL	L	−	−A
10	33	F	+	+	+	+	−	H	+		−	−	−	−		+		U	L	−	+
11	44	F	+	+	+	+	−	−		−	−	−	−	−		+		U	U	−	−A
12	28	F	O	O	O	+	−	−	−	+	−	+	+	+		+		U	U	−	+
13	27	F	−	O	O	+	−	−		++	+	+	+	−	+			U		−	+
14	39	F	−	−	−	−	−	−		+	+	+	−	−	+			U		−	+
15	31	F	−	−	−	+	−	−	−		−	−	−	−	+				U	+	+
16	2	M	−	−	−	−	−	M	+		O	+	O	O	+				U	−	+
17	34	M	−	−	+	+	−	−	−		+	+	+	−	+			U		−	A
18	32	F	2	−	−	+	−	M		+	+	−	−	−		+		UM	U	−	+
19	27	M	+	−	−	−	−	W	+		−	−	−	−	+			U		−	+
20	21	F	−	+	−	+	−	−	−		+	+	+	+		+		U	U	+	+
21	33	M	+	−	−	+	−	−	−		−	+	+	−		+		U	U	−	+
22	57	M	−	−	+	+	−	−		−	−	+	+	−		+		U		−	
23	45	M	−	−	−	+	−	−	−		−	+	+	+		+		U		−	+
24	22	M	−	−	−	−	−	−	−	−	−	+	+	+		+		U	U	−	+
25	41	F	+	−	−	+	−	−	+		+	−	−	+		+		U	UL	−	+
26	14	F	−	−	−	O	−	−	−		+	−	−	−	+				U	−	+
27	29	F	−	+	−	−	−	A		++	+	+	−	−		+		UL	UL	−	+
28	27	F	−	−	−	+	−	S	+		+	−	−	−	+			U	U	−	+
29	42	F	+	−	−	−	−	−		+	+	−	−	−	+			U	U	−	+
30	36	F	−	+	−	+	B	M³S		+	−	−	−	−	+			U	U	+	−
31	18	F	+	−	−	−	−	−	+		−	+	+	−	+			U	U	−	+
32	23	F	−	−	−	+	−	S		+	+	+	+	−	+			U		−	
33	43	M	−	+	−	+	B	W	−		−	+	+	+		+		U	UL	−	
34	13	F	−	−	−	−	−	−	+		+	+	−	−		+		U	U	−	
35	32	F	−	−	−	+	−	−		−	−	+	+		+				U	−	+
36	11	F	−	−	−	+	−	−	+		+	−	−	−	+			U		−	+
37	47	F	−	−	−	+	H	−	+		+	−	−	−	+			UM		+	+
38	30	F	+	−	−	+	−	−		−	−	+	−	+		+		U	U	−	+
39	22	F	+	+	+	−	−	−		+	−	+	+	+		+		U	U	+	
40	11	M	−	−	−	−	−	−	−	+	−	−	−	−		+		U	U	−	+

TABLE I (Cont'd).

ALGER CO.

NO.	AGE	SEX	PNEUMONIA	PLEURISY	HEMORRHAGE	LOSS OF WEIGHT	ASSOCIATION		TEMP. PULSE			COUGH	EXPECTORATION	NIGHT-SWEATS	STAGES			LOCATION		RALES	CALMETTE
							MALE	FEMALE	A. M.	P. M.	PULSE				INCIPIENT	MOD.	ADV.	RT.	LEFT		
41	33	M	+	+	+	−	U	−	−		−	−	−	+		+		U	U	+	+
42	6	M	−	−	−	−	F	−	+		−	−	−	−	+			U		−	
43	24	M	−	−	−	+	−	−	+		−	+	+	+		+		LU	U	+	
44	53	F	−	−	−	−	−	−		−	−	+	+	+	+			M		−	−
45	38	F	−	−	−	−	−	M	−		−	+	+	−		+		U	UL	+	−
46	24	M	−	+	−	−	−	−	++		+	+	+	−		+		UML	U	−	+
47	28	F	−	−	−	+	F	−	−		+	+	+	−		+		ML		−	+
48	46	M	−	−	−	−	−	−	−		−	+	+	−		+		UML	U	−	−
49	19	M	−	+	−	−	−	−	−		−	−	−	−		+		U	U	−	A
50	19	M	−	−	+	+	−	A		−	−	−	−	−		+		U	U	−	A
51	19	F	−	−	−	+	B	−	−		+	−	−	−		+		U	U	−	A
52	9	F	3	−	−	−	−	−	+		+	−	−	O	+			U		−	A
53	26	F	−	−	−	−	−	M	−		−	−	−	−	+			U	U	−	AA
54	11	F	−	−	−	−	−	M		−	−	−	−	−	+			Not given			−

The above table is largely self explanatory. Under association it will be noted that a classification has been made according to sex, the initial letter being used in each instance to designate the member of the family afflicted. Thus, in the male column B, refers to brother, H, husband, F, father, S, son, and U, uncle; whereas in the female column, M represents mother, S, sister, W, wife and A, aunt. With regard to temperature, the symbol + has been used to designate any temperature occurring in an individual of fifteen years or over and which registers between 99 and 100 degrees F. In children no temperature below 99.6 was recorded as positive. Temperatures ranging from 100.1 to 101 inclusive are recorded as + + and any temperature above 101 has been recorded as + + +. With regard to pulse in individuals fifteen years or over a pulse ranging from 90 to 120 has been classified as +; between 120 and 130 + + and above the latter point + + +. Here again in children no pulse below 110 has been classified as positive. In connection with location of lesion initials have again been used to designate the lobe of the lung affected as U, upper; M, middle and L, lower lobe. In the last column are given not only the results with regard to the occurrence of positive and negative tuberculin reactions, but the letters A and P are used to designate cases which are apparently arrested and those showing physical signs indicative of pleurisy respectively.

AGE, SEX, AND ASSOCIATION.

Among 2923 cases in which the sex was designated there were 1786 females and 1137 males. With regard to age, 764, or 26.1%, were fifteen years or less; 1041, or 35.6%, were between sixteen and thirty years inclusive; 747, or 25.5% between thirty-one and forty-five years inclusve; 282, or 9.6%, were between forty-six and sixty inclusive; and 89, or .3%, were between sixty-one and eighty years. Sixty-one and one-tenth per cent of all cases occurred between the ages of sixteen and forty-five.

Chart 1 gives the number of cases which gave a history of association with a single tuberculous member of the family. All cases in which two or more sources of infection occurred have been excluded. It will be noted that during the first fifteen years of life the source of infection in the majority

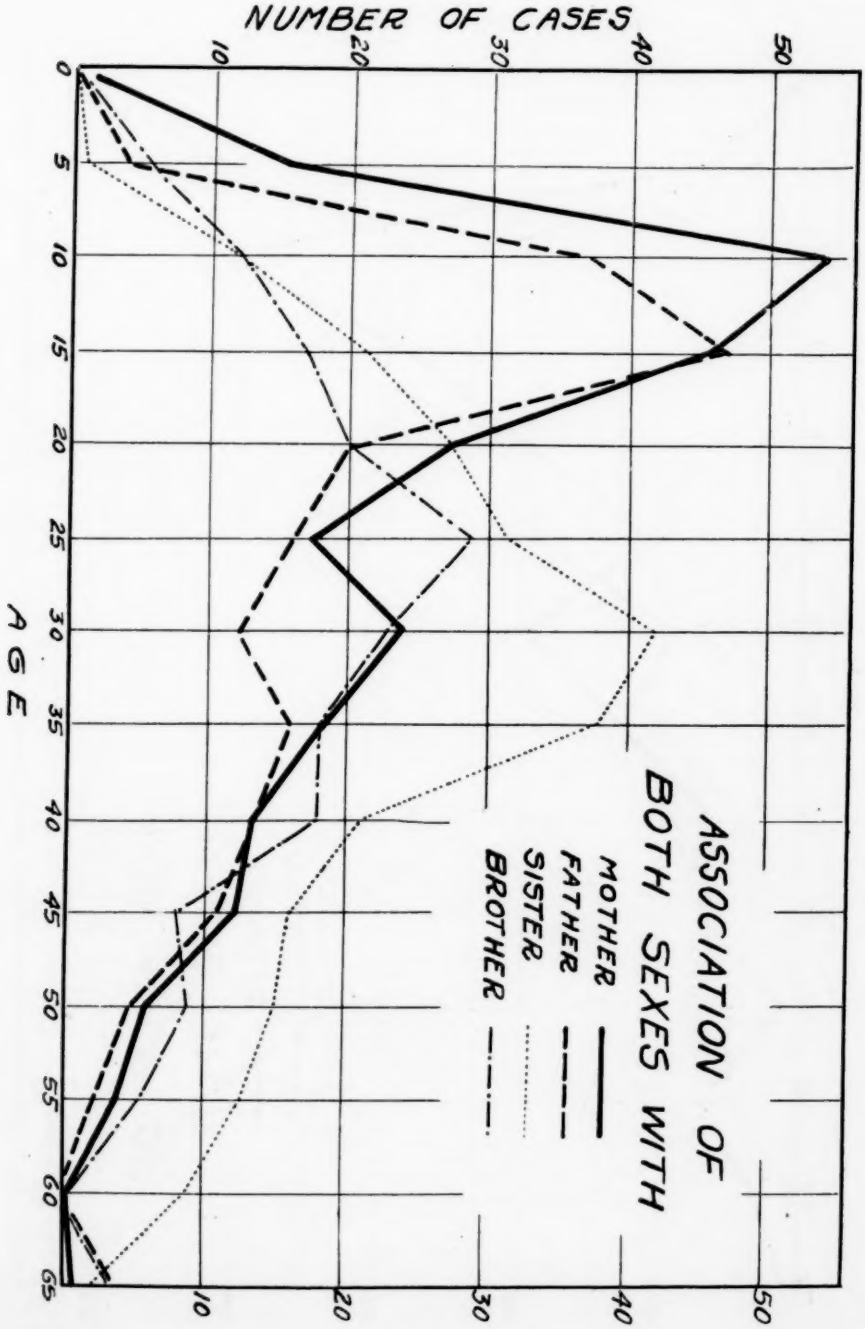

Chart 1.

of instances is either the father or mother, while beyond twenty years of age a history of tuberculosis in brother or sister predominates, the brother line reaching its maximum at twenty-five and the sister line at thirty years. The part played by intimate association is well illustrated in Charts 2 and 3.

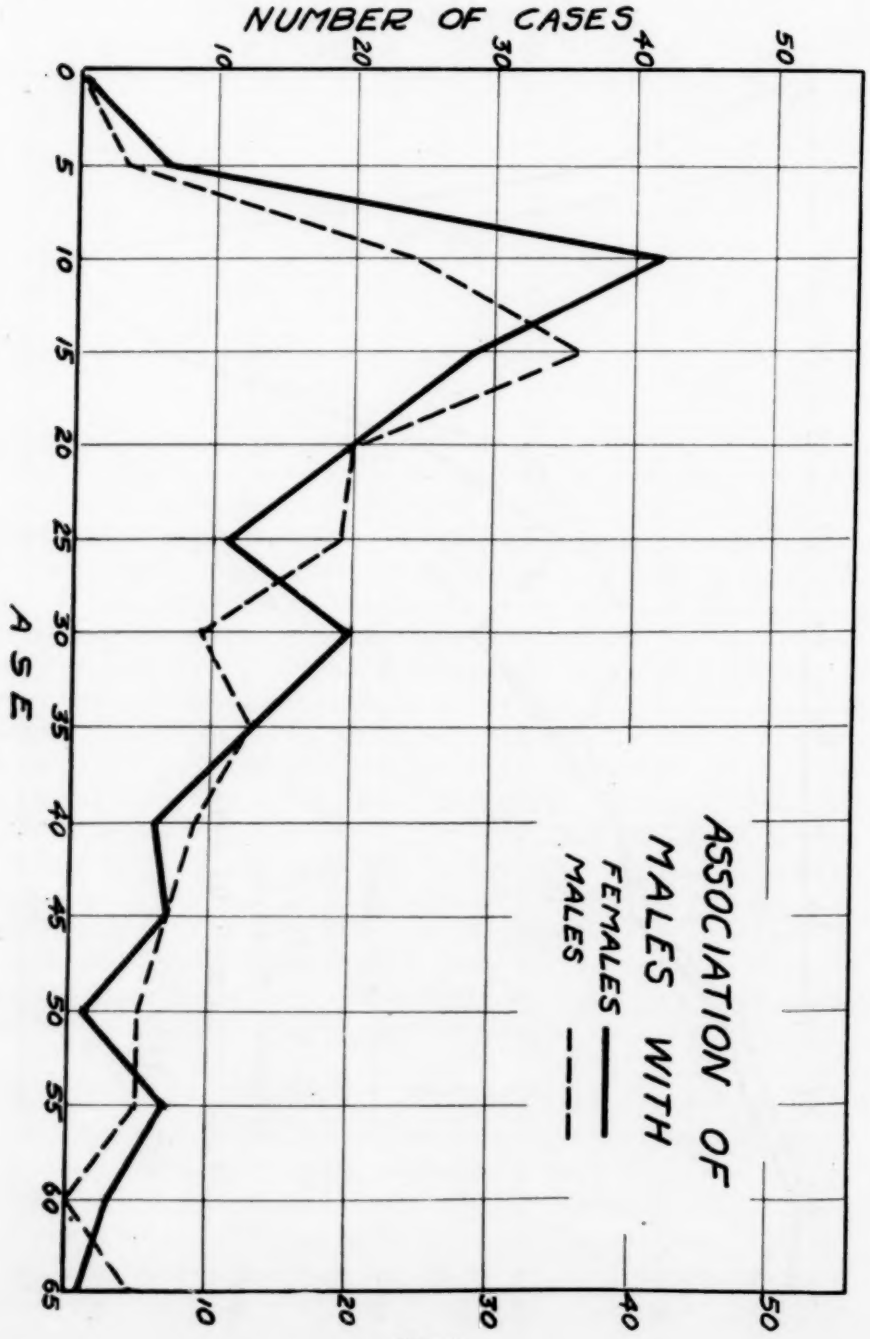

Chart 2.

Chart 2 represents the total number of males at the various ages giving a history of family association with a single tuberculous individual, while Chart 3 represents the total number of females at the various ages giving a history of family association with a single tuberculous individual. For obvious reasons

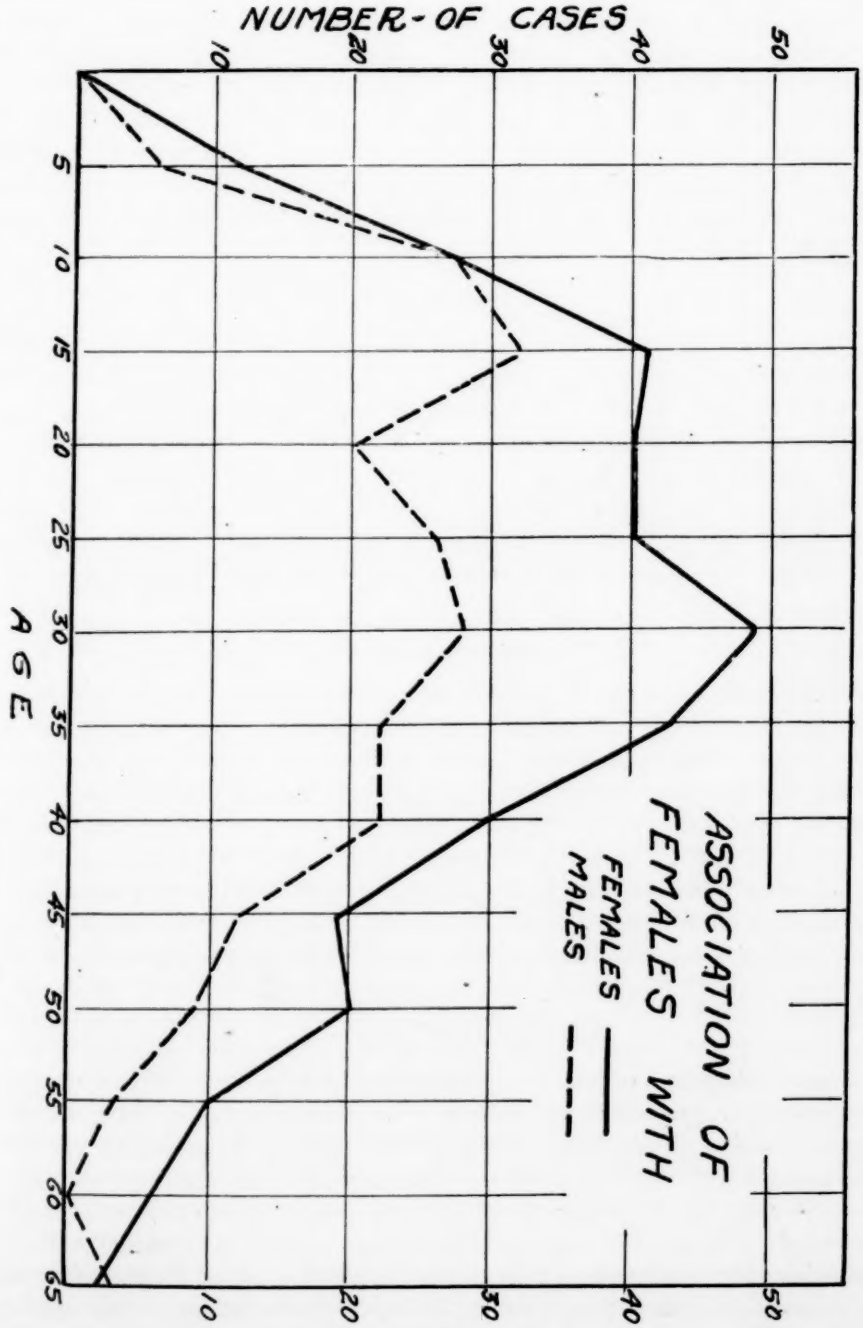

Chart 3.

individuals giving a history of tuberculosis in husband or wife have been omitted from these charts. In Chart 2 it will be noted that until ten years of age the female predominates as a source of infection, this being undoubtedly due to the close association existing between mother and child at this period of life, irrespective of difference in sex. From ten to fifteen years in boys the female line drops abruptly while the male becomes the greater source of infection. From fifteen on, the male source of infection generally predominates. In Chart 3, on the other hand, the female predominates throughout as a source of infection. It is interesting to note, however, that below the age of ten no marked difference in the two sexes is notable although here again the mother, as one would expect, represents the greater source of infection. After ten years the part played by intimate association between members of the same sex is most apparent.

PLEURISY.

Among the 2957 cases there were 605 who gave a definite history of pleuritic involvement at some time previous to, or coincident with, the examination, there being 46 cases in which no information with regard to this point was obtained. A history of pleuritic involvement was, therefore, obtained in 20.8% of all cases questioned with regard to this point. Owing to the definite character of pleuritic pain we believe that these statistics are most reliable. It is needless to state that all pleurisies associated with pneumonic attacks either as initial pleuritic involvement or as subsequently developing empyema were neglected.

HEMORRHAGE.

Among 2906 cases in which information with regard to the occurrence of pulmonary hemorrhage was obtained, 428, or 14.7%, had been afflicted at some time previous to the examination. Mere streaking of sputum was not considered, nor was the rusty sputum associated with pneumonic attacks. No case of a profuse pulmonary hemorrhage associated with a supposed pneumonic attack presented itself, although in this instance we would have been inclined to question the original diagnosis. Any amount of pure blood from a small clot to the expectoration of many ounces undoubtedly coming from the lower respiratory passages and accompanied by cough has been classified as pulmonary hemorrhage.

CLASSIFICATION BY STAGE.

The classification of cases of pulmonary trouble into various stages of the disease is always a difficult problem. We believe that such classification is of great practical importance for the information of the attending physician with regard to the ultimate prognosis, but we also feel that such a method necessarily partakes somewhat of the nature of scientific inaccuracy. The classification adopted by the National Association for the Study and Prevention of Tuberculosis has been followed as closely as possible and each individual case was classified as originally tabulated, the results obtained not being apparent until the final compilation of the complete table.

The cases have been divided into three stages as follows:

First Stage.

Incipient.

Slight or no constitutional symptoms (including particularly gastric or intestinal disturbance, or rapid loss of weight); slight or no elevation of temperature or acceleration of pulse at any time during the twenty-four hours.

Expectoration usually small in amount or absent.

Tubercle bacilli may be present or absent.

Slight infiltration limited to the apex of one or both lungs, or a small part of one lobe.

No tuberculous complication.

Second Stage.

Moderately advanced.

No marked impairment of function, either local or constitutional.

Marked infiltration more extensive than under incipient, with little or no evidence of cavity formation.

No serious tuberculous complications.

Third Stage.

Far advanced.

Marked impairment of function, local and constitutional.

Extensive localized infiltration or consolidation in one or more lobes.

Or serious tuberculous complications.

From Table II it will be seen that a classification of 2942 cases has been made according to stage. The interesting developments obtained from this classification are best demonstrated in Chart 4 which represents graphically the information obtained in table 2.

As can be seen from an examination of Chart 4 the symptoms not influenced by time of examination, as cough, expectoration, and night sweats, show practically the same percentage in the same stage at morning and afternoon clinics. From this fact we can fairly conclude that a definite degree of uniformity has been used in classification. It might be objected that the percentage of night sweats is high in the incipent cases and yet these for the most part represent cases showing but slight rise of temperature, no acceleration of the pulse, without expectoration and with physical signs limited to slight changes at one apex. Such cases cannot be classified otherwise than as incipient.

An examination of Chart 4 reveals at once most interesting variations between temperature and pulse findings in the incipient and moderately advanced cases. The most striking phenomenon is the higher percentage of cases showing a temperature between 99 and 100 degrees F. among the incipient as compared to the moderately advanced. This change, apparent even during the morning examinations, becomes pronounced during those of the afternoon. We believe that this is an indication of the fact that the true tuberculous temperature occurs most frequently in the incipient stage of the disease. It represents the rise in body temperature accompanying the specific reaction which occurs in the body of the recently infected tuberculous individual and which is frequently exhausted by the time the case becomes moderately advanced. Further evidence with regard to the fact that specific reaction occurs early in the disease is found in the results of tuberculin tests, which are conceded by all authorities to give positive reaction most frequently in the incipient stage and to disappear as the

Table II.

CLASSIFICATION	TOTAL EXAMINED	POSITIVE TEMP.		POSITIVE PULSE		COUGH		EXPECTORATION		SWEATS	
		NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
EXAMINED IN A.M.											
EARLY	747	334	44.7	316	42.3	382	51.2	258	34.5	134	17.9
MOD. ADV.	536	229	42.7	261	48.7	381	71.0	316	59.0	184	34.3
ADVANCED	89	37	41.6	65	61.8	81	91.0	78	87.6	46	51.7
EXAMINED IN P.M.											
EARLY	968	606	62.6	469	48.5	455	47.0	306	31.6	159	16.4
MOD. ADV.	524	295	56.3	283	54.0	382	72.9	303	57.8	174	33.3
ADVANCED	78	53	68.0	57	73.0	74	94.9	67	85.9	41	52.6

case progresses. Thus in Chart 6, although the total number of cases is small, there is distinct evidence that the percentage of negative conjunctival reactions increases in direct proportion to the advancement of the disease process.

When we consider the pulse, we find that instead of paralleling the temperature, the exact reverse is true. Thus, whereas the greater percentage of slight

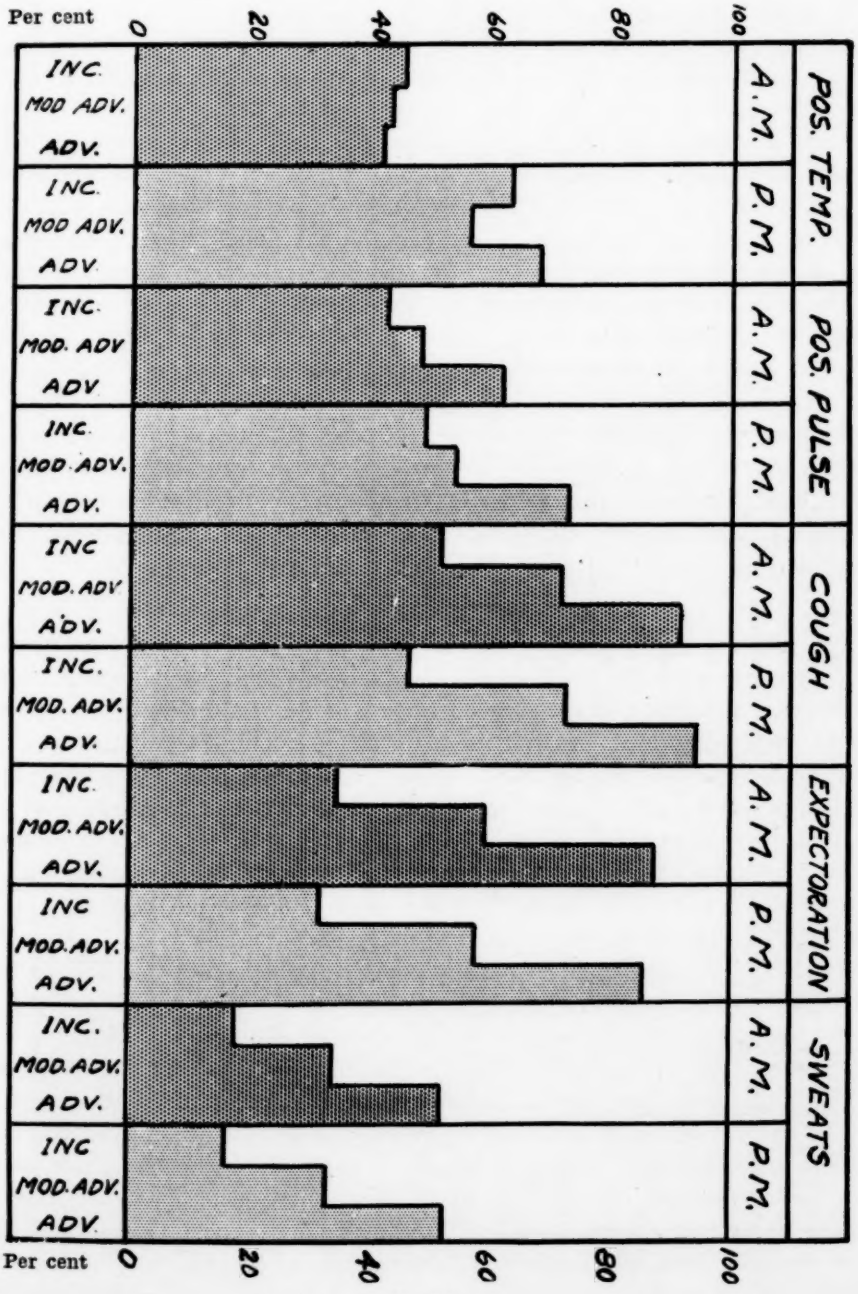

Chart 4.

febrile reactions occur in the early cases the percentage of high pulse is distinctly increased among the moderately advanced. The explanation of this discrepancy is probably to be found in the greater area of involvement and the relatively prolonged toxic action upon the myocardium in the moderately advanced cases.

EXTENT AND LOCATION OF PULMONARY INVOLVEMENT.

Of 2002 cases studied, the involvement was limited to one lobe in 990, or 49.5%; two lobes were affected in 717, or 35.8%; three in 212, or 10.6%; four in 57, or 3.8%; and involvement of all five lobes was recorded in 26, or 1.3%, of the cases.

From a study of Chart 5 it is evident that the right upper lobe is most frequently the site of disease, next in order come the left upper, the left lower, the right lower, and middle lobes. However, when we consider signs indicative of activity it becomes at once apparent that the left upper shows a higher per cent of activity than the right upper. Thus whereas râles were found associated with involvement of the right upper in only 45.6% of cases they were present in 57.2% of involvement of the left upper, in 58.2% of left lower involvement, in 60% of right lower, and in 62.4% of right middle involvement. Taking into consideration the larger number of cases of left lower involvement (483) as against those of right lower and middle, 310, and 284, respectively, we appear to have confirmation of the result obtained by Walsh who stated that in pulmonary tuberculosis the disease would seem to begin in most patients at the right apex, the subsequent extension being to the left apex and then to the base of the left lung.

TUBERCULIN TESTS.

The tuberculin tests employed by all examiners have consisted entirely of those dependent upon the local specific reaction occurring as the result of the application of tuberculin to the abraded surface of the skin or the mucous membrane of the conjunctiva. At first the majority of examiners hesitated to make use of the conjunctival test but finally, being convinced of its freedom from danger when properly applied in selected cases and its obvious superiority over the hypersensitive vaccination test, they have employed it freely. At many small places where clinics were held for a single day only, it was impossible to apply the test. However, in the larger centers where clinics were held on two or three successive days the test was extensively used on suspicious cases. The added value of the opportunity to examine the suspected individual on two successive days cannot be overestimated and frequently resulted in the obtaining of valuable positive evidence even in the presence of a negative tuberculin test. In no instance was a diagnosis made upon a positive tuberculin test alone, nor was a negative test interpreted as indicating the absence of tuberculous disease.

The record shows 751 tuberculous cases in which the results of the conjunctival test have been recorded, 583 of which were positive and 168 negative. Among these individuals, 113 gave a history of pleurisy at some time in the past and of this number 88, or 77.8%, responded positively to the test. There were 72 cases of pulmonary hemorrhage, 52 or 72.2% of which responded with a positive tuberculin test. Of the cases examined during the

afternoon clinic, 69.2% of the positive conjunctival tests showed a febrile reaction while this was obtained in only 55.2% of the tuberculous cases failing to respond to the test and examined at the same time of day.

In our preliminary report no tabulation of tuberculous conditions other

LOCATION	NUMBER WITH RALES	PERCENT OF TOTAL INVOLVED	NUMBER WITHOUT RALES	PERCENT OF TOTAL INVOLVED	TOTAL
RIGHT UPPER	893	45.6	1064	54.4	1957
RIGHT MIDDLE	177	62.4	107	37.6	284
RIGHT LOWER	186	60.0	124	40.0	310
LEFT UPPER	691	57.2	520	42.8	1211
LEFT LOWER	281	58.2	202	41.8	483

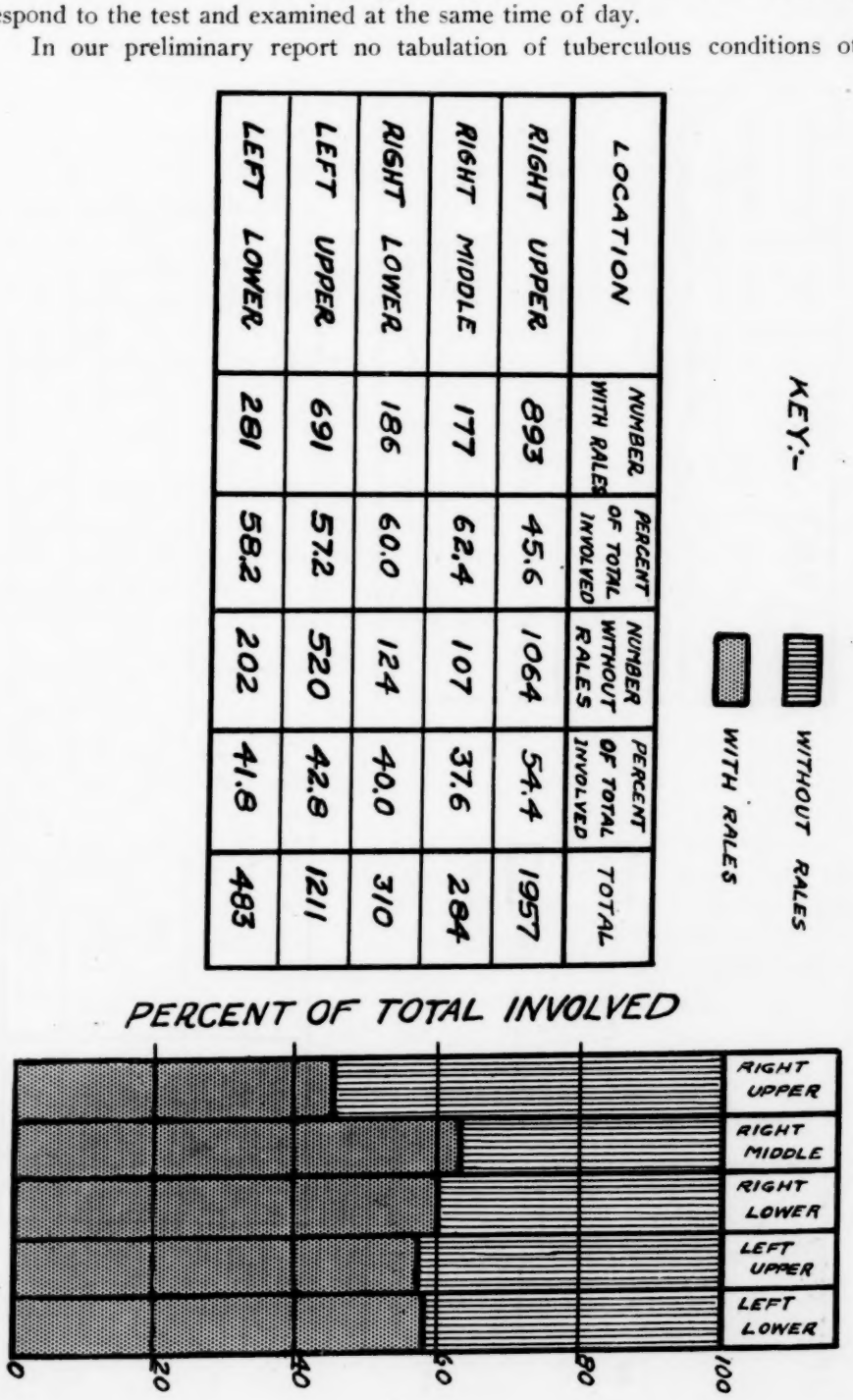

Chart 5.

than pulmonary has been presented. Cases of surgical tuberculosis without demonstrable pulmonary involvement have presented themselves for examination and cases in which the process was apparently limited to glandular involvement have been recorded. The total number of such cases is, however, comparatively

STAGE	POSITIVE CALMETTE	PERCENT OF TOTAL POS.	NEGATIVE CALMETTE	PERCENT OF TOTAL NEG.
INCIPIENT	425	72.9	107	63.6
MOD. ADV.	150	25.7	56	33.4
ADVANCED	8	1.4	5	3.0
TOTAL	583	100.0	168	100.0

AFTERNOON TEMPERATURE	POSITIVE	NEGATIVE	TOTAL	PERCENT POSITIVE	PERCENT NEGATIVE
POSITIVE CALMETTE	224	100	324	69.2	30.8
NEGATIVE CALMETTE	48	39	87	55.2	44.8

HISTORY OF	CALMETTE POSITIVE	CALMETTE NEGATIVE	TOTAL	PERCENT POSITIVE	PERCENT NEGATIVE
HEM'GE.	52	20	72	72.2	27.8
PLEURISY	88	25	113	778	22.2

Chart 6.

small at present and would not appear to justify tabulation at this time. In the complete report it is our intention to give due consideration to the above mentioned types of the disease.

That the diagnosis of incipient cases particularly has been determined with great care is evidenced from the fact that of the 2957 cases tabulated we have definite records of 751 who presented themselves at the clinic for a second examination. No single symptom or test was relied upon as definitely determining the diagnosis, but the weight of total evidence eventually decided the result. That the work has been well done is best indicated by the results obtained from the total compilation with regard to various phases of the problem, which in no way varies from the logical or the expected. Whenever a doubt has remained in the mind of the examiner, the case has not been pronounced positive, but has been classified as suspicious. In all positive and suspicious cases a copy of the physical findings has been placed in the hands of the attending physician and the patient has been advised to call upon his physician for periodic examinations and medical supervision. Thus in the case of a given individual classified as suspicious, the findings of the examiner consisting of prolonged expiration at the right apex associated with a suggestion of crepitant râles beneath the outer third of the right clavicle are furnished the attending physician who by subsequent examination of the chest, repeated if necessary, will eventually determine the tuberculous or nontuberculous character of the case. It is safe to infer that individuals, who have availed themselves of the opportunities afforded by the clinic, will in the majority of instances see that they have proper subsequent supervision.

Credit is due the medical profession of the state for their hearty cooperation in the work of the survey. Physicians generally showed great interest, attended the clinics and aided materially in the discovery of cases in their locality.

In conclusion we wish again to emphasize the fact that as a result of the survey, 2957 positive cases have already been discovered. These represent an equal number of potential sources of infection to others, some actively infectious at present; 1715 incipient cases, mostly closed, innocuous at present but representing sources of infection in the future provided they are not properly cared for. The great majority of these 1715 cases will, under proper supervision, completely recover without at any time being sources of danger to others and ultimately will take their places among the useful citizens of the commonwealth.

SPIROCHÆTES*

By Hideyo Noguchi, M.D., New York City.

Part II.

TRANSMISSION OF SPIRONEMA AND TREPONEMA TO MAN AND ANIMALS.

UNDER natural conditions the transmission of a blood-inhabiting Spironema to man or animals is effected through the bite of an infected blood-sucking insect. The transmitter in each instance is highly, if not strictly, specific, although other blood-sucking insects may also be infected by sucking the blood of an animal which is suffering from an infection with any of the pathogenic blood spironemata. These unnaturally infected ticks, bedbugs, fleas, or lice are not good transmitting agents as compared with the natural carrier of the infection. That the Spironema in such nonspecific insects can survive for some time can be shown when the disease is produced in a susceptible animal by inoculating it with the crushed material of the infected insects. It is possible, therefore, that an infection can be occasioned by smearing the excreta or crushed body contents of the infected insect over any defect of the epidermic layer of a susceptible subject. For example, in the case of *Spironema recurreutis,* both body lice and bedbugs may be infected by sucking the blood of a patient suffering from the European relapsing fever, but the lice alone can transmit the disease to the next person they bite. Bedbugs are never known to spread the infection by their bites although by crushing the infected bugs directly over a minute skin trauma (scratch, etc.) a person may become infected. A brief summary is given below of the natural intermediary hosts of different bearing spironemata, as well as certain experimental data bearing on the role of other blood-sucking insects and on the susceptibility of various animals of each Spironema.

Spironema recurrentis, the causative agent of the European relapsing fever, is naturally transmitted by *Pediculus corporis. Pediculus caputi* was found by Gonder to be incapable of transmitting the disease, although its body may contain the organisms. The common bedbugs (*Acanthia lectularia*) may likewise harbor the Spironema for as long[130, 131, 132] as sixty days, but according to the experiments of various investigators, does not spread the infection. The rat louse *(Hematopinus spirulosis)* can carry the infection from rat to rat, while the monkey louse does the same among monkeys. Breinl and Kinghorn,[133] as well as Neumann,[134] Manteufel,[135] and Sergent and Foley[136] succeeded in transmitting the infection to rats with ticks *(Ornithodorus moubata).* Schuberg and Kuhn report a successful transmission by *Stomaxys,* the blood-sucking flies.

The infection can be transmitted subcutaneously as well as *per os* in experimental animals.

Infected organs fed to rats produce the infection in these animals, as shown by Uhlenhuth and Haendel,[137] and others. Manteufel considers the uninjured

*From the Rockefeller Institute for Medical Research.
Harvey lecture delivered on February 5, 1916, at the New York Academy of Medicine.

skin permeable to S. recurrenti, and Nattan-Larrier produced the infection *per vagina, per penis,* etc., in rats, while Gozony successfully transmitted the disease also by means of subcutaneous, conjunctival, and intestinal application of the Spironema. Schellack,[138] who obtained a positive result in one out of twenty-eight experiments on rats, was able to demonstrate a microscopical defect of the skin at the point of entrance. The organism is experimentally transmissible to monkeys, and from monkeys to rats, mice, guinea pigs, and sometimes rabbits.

S. duttoni, the causative agent of the African tick fever, is normally carried by *Ornithodorus moubata,* as was recognized by Dutton and Todd,[139] and Koch.[139] The last named investigator discovered the Spironema in the ovaries four to five days after the tick had sucked the infected blood. Carter[140] confirmed this finding, while Neumann[134] found the organisms in freshly laid eggs. Hereditary infection for one or more generations was shown to occur by the study of Dutton and Todd, Wolbach,[141] and others. The tick is infectious an hour after sucking and remains so as long as 90 days (Wittrock[142]). This author always found the Spironema in the infective ticks. *Ornithodorus savignyi* has been suspected of carrying the infection,[143] and Brumpt once succeeded in infecting a monkey by this tick. Robledo holds *Argas americanus* responsible for the spreading of *S. novyi* (the American type of relapsing fever) in Columbia, but this theory calls for further investigation. According to Breinl and Kinghorn,[144] the rat's fetus may be infected through the placenta when a mother rat is inoculated with *S. duttoni.* The organism is experimentally transmissible to rats, mice, monkeys, guinea pigs, and rarely to rabbits.

As has been briefly mentioned elsewhere, Leishman, Fantham, Hindle, and others assume a granular or coccoid phase in the life history of this and allied species, and maintain that the Spironema gradually undergoes granulation when it reaches the tick's body and multiplies in the Malpighian tubules and ovaries. The tick becomes infective after an incubation of 1 to 2 days at 37°C. Hindle[70] demonstrated the infectivity of the coxal fluid in which he found numerous granules and some spironemata. This investigator thinks that the Spironema or infective granules in the coxal fluid enter the body of persons through the wound produced by the bite of the tick. The infected eggs become infective after being incubated, as was demonstrated by Hindle by injecting the crushed material into the susceptible animals; Leishman[145, 146] found numerous spiral rods in the infected tick eggs when the latter were incubated for a few days at 35°C. Schubert and Manteufel showed the infectivity of the ticks to be lost when they are kept at a temperature below 22° C., but Gonder failed to find any such difference. Marchoux and Couvy, Gleitmann, Wolbach, Wittrock, Kleine and Eckard, and others, believe that, wherever infectivity is present, there are always to be found some typical spironemata, either in the tick or in its eggs.

Spironema berbera (the North African type) is carried by *Pediculus corporis,* but not by *Argas,* the flea or bedbug[147, 148] (Sergent, Gillet, and Foley). *S. carteri* is also transmitted by body lice. In this case Mackie[149] found the organisms more numerous in the female lice than in the male; they are distributed in the mouth, stomach, and digestive tract. Mackie believes, however, that *Acanthia lectularia* sometimes carries the infection.

Among the ticks which transmit Spironema in cattle and sheep may be mentioned *Boophilus decolaratus* and *Rhipicephalus evertsi.* The virus is carried by heredity. The causative agent of chicken fever, *S. gallinarum,* is carried by *Argas persicus* under natural conditions, while other species *(Argas reflexus* and *Argas miniatus)* can transmit the disease experimentally.[138] *Ornithodorus moubata* is doubtful, as Schellack[138] failed to produce the infection while Fülleborn and Mayer[150] claim a success with this organism. Schellack was able to produce the infection in 3 out of the 15 experiments performed by him on chickens by the percutaneous application of the infected blood. Feeding fowls with infected ticks may cause the infection. The organism is experimentally transmissible to ducks, geese, sparrows, canaries, and sometimes rabbits. *S. icterohemorrhagiæ,* the causative organism of Weil's disease, has been shown by Inada and his associates to be but rarely conveyed by direct contact, but no natural intermediary hosts have been discovered. The organisms are abundantly present in the urine during the convalescent stage and they are fully virulent for guinea pigs. According to the experiments of Inada and his associates, the Spironema as contained in the liver emulsion is capable of penetrating an apparently uninjured skin of the guinea pig a short time after contact (5 minutes is sufficient to cause infection).* Therefore, it is altogether possible to infect a person through direct contact with some of the excreta of a patient. The infection can be induced in guinea pigs by introducing the infected material into the stomach after it has been previously neutralized with bicarbonate of soda. In regard to the Spironema found by Futaki and others at the site of or in glands adjacent to the rat bite, we must assume that this represents the occurrence in rats of a pathogenic Spironema which produces fever and other symptoms when transmitted to human subjects. Further investigation in this direction is most desirable. The organism is most easily experimentally transmissible to guinea pigs. Monkeys, rats, and mice are less susceptible.

Prior to Futaki's work there appeared a report by Kitagawa and Mukoyama, who also found a spironema in the inflamed tissue of the bitten finger of a woman. By transmitting the tissue into guinea pigs and white rats, these authors claim to have reproduced symptoms resembling the so-called rat-bite fever. In the smears of the kidney and liver taken from the dead animals, they found two types of spiral organisms; namely, in the guinea pig tissues, the refringens type; and in the white rat, the minute and short type. In examining the preparations kindly sent to me by the authors, I found their findings to be entirely correct, but the refringens type is more like *T. macrodentium* and a large number of bacteria, such as fusiform bacilli, and big rods, etc., were also present in the same preparations. As to the short type, one can only say that its morphology is almost indistinguishable from that of *S. muris* or *S. microgyratum.* These organisms do not agree with the illustrations and description of the Spironema reported some time later by Futaki and others. In the case of Kitagawa and Mukoyama, the local and general symptoms may have been due to a mixed infection by the oral flora of a rat.

The Spironema of relapsing and tick fevers also cause in man a well char-

*Of eight guinea pigs experimented upon, only one escaped the infection which developed in from ten to twelve days with typical symptoms.

acterized type of fever accompanied by two attacks interrupted by a period of apyrexia lasting several days. During the apyrexia the blood is free of the parasites. But it is not at all rare for the recidive to be repeated more than once. While at the highest point of the fever the organisms are most abundant in the blood, they are also present in different organs. Dutton and Todd, Breinl, Leishman, and Fantham believe that the spironemata are taken up by phagocytes within which they undergo transformation into the granular phase which in turn gives rise to the new generation of spironema. Balfour considered the intraglobular forms of *Spironema granulosa penetrans* (similar to, probably identical with *S. gallinarum)* as asexual, and the extracellular forms as a sexual phase. Fantham observed some extracellular granules which may start the relapse. Darling would hold the phagocytized spironemata within the endothelial cells of the liver responsible for the source of the recidive, as he found the organism to remain intact for some time during reconvalescence. Grabitschewsky sees in the surviving resistant specimens, which had been shielded from destruction in various organs, the progeny of the organisms producing the second attack. In the case of *S. carteri,* Mackie assumed the possible existence of an ultramicroscopic phase, as the serum taken from a patient at the apyrexic period is said to be infective in spite of the absence of any spiral forms. It may be remarked that to detect a sparse number of any Spironemata under the microscope is one of the most difficult tasks, and one is very liable to overlook the organism.

T. pallidum and *T. pertenue* are the only pathogenic varieties among the group. In the case of syphilis our knowledge is quite complete, so far as the mode of transmission is concerned. On the other hand, much still remains to be learned regarding the manner in which yaws is communicated from person to person. Probably, like syphilis, its infection is spread by direct contact with a patient or any object which, after having been in contact with a patient, harbors the live organisms, although the possibility of transmission through flies, mosquitoes and ticks is not excluded. Castellani and Chalmers[151] quote an instance in which a fly which sucked on a yaws papule infected a monkey whose eyebrow was scarified. Modder[152] assumes transmission of yaws by ticks (Argas and Ixodes) in Ceylon. It is said that vaccination and wet nursing spread the infection. In order to facilitate their entrance into the human body both organisms need only a microscopical defect of the epidermis.

After penetrating the skin or mucous membrane, *T. pallidum* elicits a local reaction characterized by the circumscribed round cell infiltration known as chancre (primary lesion), then several weeks later, about the time when the chancre recedes, it proceeds to enter the adjacent lymph glands and general cutaneous and mucous membrane tissues, producing roseola, papules, and flat condyloma. At this period the organisms invade almost every tissue, producing the so-called secondary symptoms. Periostitis, meningitis, iritis, and laryngitis are very frequently observed. One of the most constant symptoms is the Wassermann reaction in the blood serum.

After a period of several months longer, during which the secondary manifestations abate, another period known as the tertiary stage may supervene, accompanied by still deeper tissue destruction caused in the organisms than at any

previous stage. It affects skin, bones, visceral organs, cardiovascular system, and central nervous system. The disease may be progressive or marked with alternate activity and latency. Yet in the latent period repeated abortions may occur. From the time of infection until the central nervous system is affected (general paralysis, tabes), the average period of latency of the disease is from eight to twelve years. During the tertiary stage the lesions are often gummatous and affect the connective tissue muscles and blood vessels, while in cases of general paralysis and tabes the parasites diffusely pervade the parenchyma.[153, 154, 155, 156] This form is a syphilitic parenchymatous encephalomyelitis. In acquired syphilis *T. pallidum* has been demonstrated in every syphilitic condition. It was first demonstrated in the primary and secondary lesions by its discoverers, Schaudinn and Hoffmann; in liver gumma, by Schaudinn; in aortitis, by Wright and Richardson,[157] Schmorl,[158] and Reuter;[159] in arteritis cerebralis, by Bender;[160] in heart muscles and pancreatitis, by Warthin;[161] in adrenal glands, by Hoffmann,[162] Jacquet and Sézary;[163] in nephritis, by Hoffmann;[164] in the cerebrospinal fluid, by Hoffmann,[165] Nichols and Hough,[166] and Sézary and Paillard;[167] in the blood during the secondary stage, by Uhlenhuth and Mulzer;[168] in paresis, by Graves;[169] in interstitial keratitis, by Igersheimer;[170] in cerebral gumma, by Dunlap;[171] in the paretic brains, by Noguchi and Moore,[172] Marinesco and Minae,[173] Levaditi, Marie and Bankowsky,[174] Mott, Rosanoff,[175] Tomasczewski and Forster,[176] Wile,[177] and others; in spinal cord, by Noguchi,[178] Versé,[179] and others. It should be mentioned that the first demonstration of T. pallidum in sections of tissues from acquired syphilis was accomplished by Bertarelli and Volpino[180] by means of their silver impregnation method which has since been superceded by a similar procedure amended by Levaditi.

In congenital syphilis the number of organisms present in the different organs and in different fetuses varies greatly. In some it may be extremely tedious to demonstrate the organisms, in others the whole fetus may be thickly interwoven with the intertwining nets of treponemata. The favorite site of invasion is the liver and skin, although stomach, intestines, adrenals, kidney, spleen, heart muscles, pancreas, bone marrow, lymph glands, thymus, testes, ovaries, and brain have been shown to contain the parasites, even in large numbers in certain instances.[181] The placenta and navel cord are also affected. For the first demonstration of the organisms in congenital lues, we are indebted to Levaditi,[182] who introduced his well known silver impregnation method for this study. According to personal experiences in connection with syphilitic infants who lived several days after birth, the number of pallida present was always very small and it sometimes required many hours' search to find a single specimen. A striking difference between syphilis and yaws is the absence in yaws of visceral affections and of the nervous involvement. Much yet remains to be investigated with regard to the relationship between syphilis and yaws, the causative agents of which bear so great a morphological, and, to a certain extent, a biological resemblance toward each other.

The transmissibility of syphilis to animals was long the subject of study by earlier investigators, but the first conclusive experiments in this connection were furnished by Metchnikoff and Roux[183] who succeeded before the discovery of

T. pallidum by Schaudinn in producing the primary and secondary lesions in the chimpanzee. It was also shown that these lesions were transferable to further series of animals. Immediately after the discovery of *T. pallidum* in human syphilitic tissues, Metchnikoff and Roux found the same organism in experimental syphilis, thus closing up the first link of the chain of evidence which was to prove the specificity of the organism for syphilis. They also infected macacus monkeys with the virus derived from the chimpanzee. Soon afterward Schultze[184] and Bertarelli[185] produced syphilitic keratitis in rabbits, while Parodi[186] selected the testes (intratesticular) to transmit the human strain to the rabbit. This work has been extended and elaborated by later investigators, particularly by Neisser,[187] Hoffmann, Loehe, and Mulzer,[188] Uhlenhuth and Mulzer,[189] Grouven,[190, 191, 192] Nichols,[193] Tomasczewski,[194, 195, 196, 197] and others. In monkeys the best site for inoculation is the eyebrow, while in rabbits intratesticular, scrotal, intraocular and intracardial inoculations were recommended. For the purpose of keeping up the pallidum strain, the intratesticular mode is preferable, especially when it is desired to obtain a pure material for cultivation (Uhlenhuth, Noguchi); but in case of utilizing the lesions in order to determine the effect of a therapeutic agent, Hata[128] recommends the scrotal chancre method introduced by Tomasczewski,[194] wherein he is supported by the experience of Brown and Pearce.[198] With the purpose of causing a generalized syphilis in the rabbit—which animal is usually refractory to the systemic pallidum infection—Grouven reports the intracardial introduction of a large quantity of the pallidum in half-grown rabbits. My own numerous attempts to produce generalized syphilis by this method completely failed, probably owing to the difference in the strains employed. It may be mentioned, however, that with certain pallidum strains symptoms similar to human secondaries or tertiaries could be produced by means of intravenous or intratesticular inoculation. I have a few times observed iritis, keratitis, and squamatous or ulcerative skin lesions, in the last of which the pallidum could be demonstrated. Nichols and Hough[166] were the first, however, to isolate a strain from a case of nervous recidive which constantly invaded the cornea, even before the local symptoms (testis) commenced to appear. This strain has most persistently caused keratitis and choroidoretinitis in rabbits. This phenomenon led Nichols to assume that the strain possessed a highly invasive character.[199] The patient from whom this strain was obtained died several months later of a rapidly progressive form of meningo-encephalitis, and the duration of the infection (from the time of the chancre to death) was very short. Nichols, therefore, considered that this case was explained by the character of the strain. Reasoner[200] also obtained a strain from a rapidly fatal case which was characterized by the early production of choroiditis in rabbits. In some rabbits the choroiditis was the only symptom in spite of its being introduced into the testis or vein. While studying ten different strains of *Treponema pallidum* I was once struck with the constancy with which the various types were associated with certain distinct characters of the lesions produced in rabbits. For example, I could discern the differences among different strains in the width, length, and number of curves to a given space, etc. I divided these strains into a thick, a thin, and a medium type. The differences were great enough to enable me to

identify different strains as belonging to any one of the three types.[201] In a series of passages covering a period of about one year and a half, it was found that the thin type produced a soft, diffusely swelling orchitis within 10 to 14 days and did not form any definite nodules even after six weeks. On the other hand, the thick type produced a hard, circumscribed nodule of varying size within about six weeks. Its development was unusually slow and the nodule remained for several more weeks. The character of the syphiloma produced by the medium type was a large, moderately firm orchitis, which started to be palpable at the end of about four weeks. As will be mentioned later, these three type strains were cultivated in an artificial medium and were found to retain their morphological characteristics unchanged. Again, my experience with the two paretic strains of Treponema pallidum transmitted from human brains to rabbits' testicles (using 36 rabbits for six specimens of brains) showed me that they were of lower virulence than the ordinary chancre strains in my possession, as they required 97 and 102 days respectively before the lesions could be definitely demonstrated.[119] With the usual skin strains four weeks' incubation is the average. Wile[177] recently reported a successful transmission of the pallidum from the living paretics to rabbits' testicles (using one rabbit for six specimens of brains) in which the lesions appeared within 14 days, and he concluded that the paretic strains were more virulent than the ordinary strains. It may be recalled that the persistent endeavors to produce syphilitic orchitis in rabbits by means of the paretic brains was not limited to a few investigators. Tomasczewski and Forster,[202] who in 1913 performed the Neisser-Pollack puncture on 62 cases at the University Institute in Berlin and found numerous examples of the motile pallida in 29 cases of the removed material, inoculated a large number of rabbits. Their results were uniformly negative. Marie, Levaditi and Bankowsky, Marinesco, Mott, and others also failed to obtain a single positive result. Another interesting feature characteristic of the strain obtained by Wile[203] is the readiness with which it at once adapted itself to an artificial culture medium, generally known to be unsuitable for the purpose of obtaining an initial growth with any strain which is transmitted to the rabbits' testicle. As I have pointed out on several previous occasions, a solid medium consisting of fresh tissue, ascitic fluid and agar, is not suitable for such a purpose, and this fact has been confirmed by numerous investigators (Uhlenhuth, Zinsser and Hopkins, and others).

It will be incomplete if we pass on without reviewing the interesting observations of Graves,[169] who succeeded in infecting a certain number of rabbits by injecting the blood of paretic patients. Graves obtained the blood in small glass ampules (sterile) which were immediately sealed. The different specimens were put in an incubator at about 37°C., and after a number of days the contents of these ampules were inoculated into the testicles of rabbits. Although the majority of the inoculations were negative, he found a strain developing in one of the animals. Morphologically, the organisms were the typical pallidum and produced local as well as generalized reactions (ulcerative lesions near the nostrils, anus, prepuce, vagina, etc.) wherein the organisms were demonstrated. The incubation period of average duration is about three to four weeks. This strain was characterized by the early appearance of keratitis in rabbits. The observation of

Graves furnishes us with a problem, viz., the fact that the sample of paretic blood sealed in a tube and left many weeks and months at an indifferent temperature was still capable of infecting a rabbit with such extreme severity. Yet Graves never succeeded in cultivating any strain of such samples; neither could he demonstrate the presence of any definite pallidum. Therefore, as Graves seems to think, *Treponema pallidum* must possess a stage of its life cycle which is still little understood by us. Can there be a resistant form which remains dormant for years until favorable conditions are secured? Clinical evidence has suggested this idea to certain syphilologists. Personal experiences with cultivated strains of *Treponema pallidum* do not justify my assuming the existence of a resistant form, except for the fact that the pallidum under cultural conditions is one of the most viable organisms. In suitable media it survives over one year when kept at 37°C., and it is not impossible that under naturally favorable conditions it may remain dormant for many years.

Several other animals besides monkeys and rabbits are susceptible of the disease. In dogs and sheep (Bertarelli, Hoffmann, Brüning), in guinea pigs and goats (Bertarelli), and in cats (Levaditi and Yamanouchi) specific keratitis has been produced. Testicles of guinea pigs (Truffi, Tomasczewski, W. H. Hoffmann, Uhlenhuth and Mulzer) and goats (Uhlenhuth and Mulzer) are also susceptible to infection by *Treponema pallidum*. Schereschewsky reported a scrotal chancre experimentally produced in a pig.

Treponema pertenue has been successfully transmitted into monkeys by Neisser, Baermann and Halberstädter[204] with skin papules, and by Castellani[205] with a punctate of the spleen of a patient. Nichols[206] transmitted it from man to *Macacus rhesus* and then from the latter to the rabbit. In the rabbit's testicle it produces a hard induration much like a syphilitic chancre. The parenchymatous orchitis finally extends over to the tunica and scrotum in which an extensive ulcerative indurated lesion results. When inoculated to the eyebrows of *Macacus rhesus*, the yaws organism produces highly destructive ulcerative papules which may remain unhealed for many months. In these lesions *Treponema pertenue* can easily be demonstrated. Halberstädter[207] observed a generalized eruption in an orang-outang 4 months after inoculation. In lower monkeys the lesion remains localized and heals in from three to thirteen weeks; sometimes it may result in a serpiginous recidive which tends to become diffuse.

FILTERABILITY OF SPIRONEMA AND TREPONEMA.

According to the experiments of Novy and Knapp,[18] *Spironema recurrentis* and *S. duttoni* pass in one form or another through the pores of Berkefeld filters, the walls of which were either previously shaved off to a thickness of 1.4 to 2.5 mm. or left intact (4.2 mm.) as the filtrates obtained by this procedure were able to produce in susceptible animals a slight infection accompanied by sparse spironemata appearing in the blood. The scarcity of the organism is ascribed to the presence of immune substances in the filtrate which was simultaneously introduced. Breinl and Kinghorn[133] obtained similar results with unmodified filters. In neither instance did the infective filtrates contain the Spironema in its spiral form. Their experiments tended to suggest a filterable phase in the life cycle of

this organism. Todd and Wolbach[208] report the successful filtration of the organism through the Berkefeld filters N and V, by pressures of fifty to ninety pounds to the square inch. Under these conditions the organism traversed the tortuous pores of the filters and was seen to have retained its usual spiral form when it appeared in the filtrate. Todd succeeded in finding the organisms in the filtrates of one experiment into which the control bacteria did not pass. Wolbach found the Spironema in the act of passing through the pores by preparing a thin section of the filter which had been employed for the filtration. He is of the opinion that the infectivity of a filtrate is due to the presence of the regular organism and not to that of filterable granules, as is assumed by others. C. Fraenkel failed to obtain an infective filtrate with any filtrates whatever.

Spironema icterohemorrhagiæ was found by Inada and his associates[79] to pass through the Berkefeld filters, grades V and N. Out of 28 experiments the filtrates were found to be infective for guinea pigs fifteen times. It is not stated whether the filtrate contained the Spironema in a regular form. Huebener and Reiter[209] also report the filterability of the virus of Weil's disease prevalent in Germany. Since they claim a spiral organism, *Spirochæta nodosa*, found by them to be the etiological agent, the same organism must be considered filterable. It passes through the Berkefeld filters V and N. As mentioned elsewhere, *Spironema nodosum* (*Spirochæta nodosa*) is probably the same organism as *Spironema icterohemorrhagiæ* (*Spirochæta icterohemorrhagiæ*) discovered a year earlier by Inada.

Treponema pallidum and Treponema pertenue are unable to pass through any bacteria-proof filters when filtered by the usual processes [application of a vacuum or a positive (compressed air) pressure]. Metchnikoff, Klingmüller and Baermann,[210] Casagrandi and de Luca,[211] and many others, established this fact in the case of syphilis, and Castellani in the case of yaws. On the other hand, the pallidum can grow through the pores of the Berkefeld filters,[57] grades V and N, and appear in the filtrate when provided with favorable cultural conditions for several days. On the fourth day the young forms commence to appear in the fluid which collects in the empty tube that is fitted up to receive the drops that fall by spontaneous diffusion without suction or pressure. This phenomenon, which was first noticed and utilized by myself when obtaining a pure culture from mixed cultures, has since been confirmed by Nakano[212] and others.

There are yet other spiral organisms which are of great interest from the standpoint of filterability. Thus, Wolbach and Binger described *Spirochæta elusa*[213] and *Spirochæta biflexa*,[214] which they obtained in a filtrate of stagnant water taken from the shores of a fresh water pond in the vicinity of Boston. The first was cultivated but the second was not. With the culture of *S. elusa*, which measures about 0.5 μ wide and 20 μ long with an average of six to eight curves, they were able to demonstrate the organism in the filtrate within about fifteen minutes. The filtration was made by suction with the Berkefeld filters, V and N. The organism is provided with one terminal flagellum at each end and is extremely motile. *Spirochæta biflexa* is a much more delicate organism. Another filterable organism, morphologically considered a "Spirochæta" in the loose sense of the term, was obtained by Wolbach and his asso-

ciate, from human feces. In explaining the filterability of these rather coarse spiral organisms, which are larger than many bacteria, Wolbach considers their plasticity to be one of the important factors.

CULTIVATION.

Only a comparatively limited number of "spirochætes" have been cultivated on artificial media. Of the free-living varieties *Spirochæta plicatilis* was cultivated by Zuelzer[22] in a flask containing ¾ liter of stagnant lake water and ¼ liter of water to which a certain amount of hydrogen sulphide had been added. According to this procedure the flask is hermetically (anaerobically) sealed after the inoculation, and hydrogen sulphide occasionally introduced. The role of H_2S is to produce sulphur by oxidation, ($H_2S + O = H_2O + S$). By this means the organism can be kept in culture for an indefinite period. Wolbach's *Spirochæta elusa* was cultivated on a hay infusion (aerobically) where it propagates indefinitely. This organism is not allied to Ehrenberg's organism, but appears more like a Spirillum. No culture has been obtained of the molluscan cristispira. Of the Spironema group several varieties have been cultivated. Attempts at the cultivation of *Spironema novyi* by Norris, Pappenheimer, and Flournoy[215] were partially successful in that these investigators were able to notice a definite multiplication of the organism in human or rat citrate blood at room temperature within 24 hours, a second generation cultivated in a similar medium bringing about the same increase the next day. A third generation was not obtained. Occasional multiplication of the Spironema in a defibrinated blood had previously been shown by earlier investigators (Lachmann, Albrecht, Gerhardt). Cultivation of the blood Spironema in the strict sense of the term was achieved by the writer[216, 217] for the first time by employing a culture medium containing a piece of fresh tissue (rabbit's kidney, etc.), and ascitic fluid (10 to 12 cm. deep). This medium provides a condition that I proposed to designate as an *aerotropic anaerobiosis;* that is, a strictly anaerobic state is produced around the base of the fresh tissue, while the top of the ascitic fluid column has access to a certain quantity of oxygen. The whole medium may be covered with a layer of sterile paraffin oil in order to prevent evaporation of the fluid and this regulates at the same time the amount of oxygen admitted.

When the medium is inoculated with a minute quantity of the Spironema-containing blood and then incubated at 37°C. the organism multiplies steadily until every field will show numerous motile specimens which may occur singly, in pairs, or in chains of three or more individuals. The height of multiplication is reached within four or six days, and a sudden degeneration of the organisms sets in on the seventh to the ninth day. By making subcultures on the fourth or fifth day the culture can be carried on indefinitely. It was found that the success or failure greatly depends upon the suitability of the ascitic fluid samples. A sample which forms a loose fibrin web with the fresh tissue (rabbit's kidney) within 24 hours at 37°C. gives the best empirical results. The addition of glucose or peptone seems to hinder the growth of the Spironema, and sterilization by filtration or fractional heating also impairs the nutrient value of the medium. Invasion of the culture by any other bacteria quickly destroys the culture; in other

words, no mixed culture has been obtained. So far I have been able to cultivate *S. recurrentis, S. novyi, S. duttoni, S. kochi* and *S. gallinarum* by the same method. Plotz[218] successfully applied this method in order to obtain a culture directly from patients in Bulgaria suffering from the European relapsing fever. According to Hata,[219] instead of the fresh tissue and ascitic fluid, a medium consisting of a piece of blood coagulum and serum of the horse may be satisfactorily used for cultivating *S. recurrentis*. At the temperature of 35°C. the culture in this medium remains actively motile for a month (Hata). None of the spironemata causing septicemia in man or birds have been cultivated on a solid medium, and nothing is known about such colonies. In a fluid medium they produce a diffuse opalescence, but no definite change of the medium is noticed. No odor or gas or change in the reaction has been detected in the culture. Their virulence remains unattenuated for many generations of artificial cultivation.

From the oral cavity *S. vincenti* and several spiral organisms have been obtained in pure cultures by various investigators. Tunnicliff[220] considers *S. vincenti* and *Bacillus fusiformis* to be identical, but different phases of development and under varying conditions. All these organisms are strictly anaerobic and can be cultivated by the usual anaerobic methods on solid media (glucose agar with or without animal proteins). They form definite colonies comparable to any other bacteria and some are putrefactive or acid-producing organisms. I am inclined to regard them as allied to Spirillum rather than to Spironema.

Spironema icterohemorrhagiæ has been cultivated by Inada and Ito in the same medium as was originally employed by the writer for the cultivation of *S. recurrentis, S. duttoni, S. gallinarum*, etc. Ito[221] later succeeded in cultivating the organism on agar or gelatine containing human or guinea pig defibrinated blood in the ratio of equal parts or one to two of blood and agar or gelatine. The organism is said to grow readily on these media at a temperature of 26° to 37°C. A good growth takes place even at room temperature. Characteristic cultural features, such as gas or odor production, colonies, turbidity, etc., have not been recorded. In the fluid as well as in the solid media no visible growth was obtained. The culture is said to remain virulent for many generations and live over one month in a solid medium when kept at about 15° to 26°C.

In proportion to its clinical importance, *Treponema pallidum* has ever since its discovery in 1905 been the subject of correspondingly more numerous investigations. Volpino and Fontana[222] observed a temporary increase of the organisms after a piece of syphilitic tissue had been put in human serum or defibrinated blood and then incubated at 37° C.; but no culture was obtained. Lebailly[223] claims to have kept alive the pallida in the syphilitic fetal tissue for 15 days when put in human serum at 37°C. Levaditi and McIntosh[224] inoculated the inactivated human serum with the expressed serum of a syphilitic lesion of a monkey containing a few pallida, and, after sealing it in a collodion sac, introduced it into the peritoneal cavity of a monkey. It was taken out after a month and was found to contain numerous motile pallida along with certain contaminating bacteria. The contents of the sac cultivated *in vivo* could be successfully transferred from one sac to another for many passages with the same result. The impure pallida cultivated by this method were avirulent for monkeys. Mühlens

and Loehe[225] failed to confirm the above findings. In 1909 Schereschewsky[226, 227] claimed to have succeeded in starting an impure culture of *Treponema pallidum* by implanting a semisolidified, clear horse serum with a piece of chancre or condyloma inserted several inches below the surface. The serum commenced to liquefy around the tissue and within several days more liquefaction took place. On examining the fluid or solid medium about the tissue he found very numerous actively motile spiral organisms resembling the pallidum. Some of them were coarser and less regularly curved and looked like *S. refringens*. An enormous number of cocci or bacilli were also present. The culture gave off an intensely offensive odor. Subcultures were carried on indefinitely. The impure culture was avirulent for experimental animals. About the same time Mühlens[228] obtained a pure culture of an organism from a syphilitic lymphadenitis of man by first using Schereschewsky's medium and then transferring the culture to another kind of solid medium consisting of horse serum and agar. In the latter he succeeded in purifying the Treponema from the contaminating bacteria. Notwithstanding the fact that the organism was derived from a material in which the pallidum would be the only small Treponema and in spite of its great resemblance to the pallidum, Mühlens' culture has certain characteristics which, as will be seen later, render the organisms distinguishable from the pallidum cultures which were obtained by others (Noguchi,[229, 230] Sowade,[231] Tomasczewski,[232] Bæslack,[233], Zinsser, Hopkins, and Gilbert.[234] Thus the organisms isolated by Mühlens were avirulent, produced a strong odor, and could grow from the beginning in a horse serum agar without the addition of any fresh tissue. W. H. Hoffmann[235] (1910-1911), a coworker of Mühlens, obtained several strains which were identical with that of Mühlens, except for the fact that he was able to produce in the rabbit's testicle a somewhat acute or subacute inflammation by injecting a large quantity of solid culture.[236] His description of the experiments leaves the syphilitic nature of the lesion indefinitely established. The extract of the organism acted as an antigen in the Wassermann reaction, as was also shown by Schereschewsky[237] in the case of his impure culture; but Mühlens as well as Schereschewsky obtained similar results when extracts of other bacteria were used. Recently Zinsser and Hopkins have confirmed the nonspecific nature of so-called antigens in this type of complement fixation. Bruckner and Galasesco[238] (1910) and Sowade[231] (1911) reported the successful inoculation of rabbits by means of their impure cultures (Schereschewsky medium) given intratesticularly and intracardially. Sowade claims to have produced generalized syphilis by the intracardial injection into half grown rabbits.

During 1910-1911 I was engaged in cultivating *T. pallidum*. Unlike the previous investigators, I had chosen the testicular syphiloma of rabbits as the material for cultivation for the reason that this material affords a constant and unlimited supply of a practically pure pallidum and as many strains simultaneously as one desires to try. Besides the rabbit strains being already acclimatized to the animal, this would more readily take when a culture derived from this source is to be tested for its virulence. After unsuccessful attempts to cultivate the pallidum in all the various media, previously reported suitable for cultivation of the pallidum, and a large number of culture media and conditions having also

failed, the following two methods were found to yield a positive growth of the organism on an artificial medium. As has been mentioned elsewhere, neither method is a perfect one and only a limited percentage of attempts is ever successful. The inconstant results are due partly to the different resistance offered by various strains to the artificial cultivation and partly to certain still unknown factors which enter into the composition of the media. At all events, the greatest difficulty in cultivating the pallidum is to obtain the first growth. As the number of generations increases the organism acquires an easier growth, and after a period of years of life in the culture, the organism becomes quite saprophytic and may grow even without the addition of fresh tissues. The strict requirements demanded by anaerobiosis and by the reactions and compositions of the media become more and more lax, until the culture may adapt itself to a great many cultural conditions. The two methods above mentioned are (1) a fluid medium consisting of a suitable sample of ascitic fluid or sheep serum water (Hiss) with the addition of a piece of freshly removed kidney or testicle from a normal rabbit; and (2) a solid medium consisting of a mixture of ascitic fluid and agar with the addition of a piece of fresh tissue as above described. The use of the fresh tissue seems to offer twofold advantages: first, as an oxygen absorbent as originally recommended by Th. Smith,[239] and secondly, as a source of nutrient substances needed for the pallidum. The first method (fluid medium) is applied exclusively for the cultivation of the testicular pallidum from rabbits, and the second (solid medium) is only used to cultivate the impure material derived directly from human syphilitic tissues. The first method requires an anaerobic apparatus, as a complete removal of oxygen from the atmosphere in which the cultivation is to be carried out is essential, while for the solid medium a layer of sterile liquid paraffin poured on the top of the culture medium suffices to prevent evaporation and possibly to minimize the diffusion of oxygen into the medium. The requisite anaerobiosis is produced by the fresh tissue which lies at the bottom of the tube. I shall not enter into any technical details, but suffice it to say that nearly a dozen strains were obtained within the last few years by the use of these two methods. The strains obtained by means of the fluid medium remained for many generations unadaptable to the solid medium to which they finally grew. On the other hand, the strains grown on a solid medium could readily be made to grow when suitable conditions were provided.* Impure pallidum cultures in a fluid medium can be purified by allowing the pallidum to grow through the pores of a Berkefeld filter. Before the associating bacteria pass, the pallidum will appear in the filtrate (by gravitation) probably on the fourth or fifth day. Some of the strains of *T. pallidum* obtained by these methods were virulent to rabbits and monkeys when tested within a few months. The lesions produced were typical in every respect, although once the organism had entered the animal body it resisted recultivation just as much as before the first cultivation. In this respect they differ from the strains of W. H. Hoffmann,[236] who was able to cultivate the organisms back from the lesions into the horse serum agar without the addition of any tissue. His strains produced a strong offensive odor when recultivated. The strains cultivated in my laboratory did not and still do not give any

*Fluid medium method.

offensive odor such as is described by Mühlens and W. H. Hoffmann. As has been stated no growth could be obtained without the aid of fresh tissue during the first year after these strains were isolated. Nor could they be induced to grow on a plain horse serum agar of Mühlens or semicoagulated horse serum of Schereschewsky. Since attention had been called to the differences which existed between the cultures of Mühlens and Hoffmann and my own cultures, later investigators gave special attention detecting any possible production of a peculiarly offensive odor. Sowade, Tomasczewski, Bæslack, Nakano, Zinsser, Hopkins, and Gilbert failed to find any such characteristic odor in their strains. Erich Hoffmann[240] considers that the cultures of Mühlens and W. H. Hoffmann either contained the pallidum and a second odor-producing organism, or were not *Treponema pallidum* at all, since there exist certain pallidum-like, easily cultivated saprophytic treponemata which in pure cultures produce a strongly offensive odor *(T. microdentium, T. mucosum, vide infra).*

That the cultivated strains of *Treponema pallidum* gradually become tolerant to various media and conditions has been strikingly demonstrated by the recent investigations of Zinsser, Hopkins, and Gilbert.[234] Thus the investigators found that a pallidum strain which they had isolated by the original fluid culture method, described by me, gave a good growth after the tenth generation in fluid media containing different kinds of autoclaved tissues of rabbits and a mixture of slightly acid meat infusion broth with heated sheep serum. This strain likewise grows well in symbiosis with *staphylococci, streptococci, Micrococcus candicans* and *Bacillus fecalis alkaligenes* added to the sheep serum agar mixture without tissue. Addition of dead staphylococci had the same effect as symbiosis. The gelatinized horse serum or sheep serum with or without the tissue proved to be a good medium for the growth of this strain. They have obtained the same results with two other strains which were isolated in my laboratory several years ago. The fact that during the first few years after isolation all my pallidum strains failed to grow on various media similar to those now successfully used by Zinsser, Hopkins, and Gilbert seems to indicate that rigid parasitic properties of the organism have gradually deteriorated due to the artificial cultural conditions, some undergoing the changes more abruptly than others. It is not at all improbable that in time the saprophytized strains of *Treponema pallidum* will adapt themselves to still simpler ordinary culture media. It is to be desired that efforts be directed toward improving the condition under which the organisms can be kept as nearly natural as those in living tissues, since the results obtained with completely denatured material might be quite different from those derived with the less modified material.

In summing up the pallidum cultivation one may say that the methods hitherto proposed are still imperfect and that much patience is still demanded in order to isolate a strain. Some strains remained persistently uncultivatable in my hands. The strains isolated by Mühlens may have been *Treponema pallidum;* but there was no way of proving this, as his culture, which was avirulent, possessed certain properties inconsistent with those of the pallidum as subsequently defined by other investigators. The first instance, therefore, of a successful cultivation of *Treponema pallidum* was that which was carried out at the Rockefeller Institute in

1910-1911, in which were brought out not only the demonstration of the pathogenicity of the organism isolated, but also the studies of other biological characteristics of the culture.

Treponema pertenue[241] was also successfully cultivated in 1911 by the same method as that given for the cultivation of the pallidum. The material used for this work was in the form of a testicular lesion of experimental yaws in the rabbit and was supplied by Captain Nichols. The organism possessed the same cultural characteristics as the pallidum, but it was probably slightly thicker and less regularly curved. Preliminary attempts to produce lesions in rabbits ended negatively, and the strain was lost before further comparative studies could be undertaken.

Several spiral organisms were isolated from unclean lesions around the genital region. *Spironema refringens*[242] and *Treponema calligyrum*[104] (from a condyloma) were cultivated by me in the pure state by methods similar to those used for the pallidum and pertenue. Levaditi and Stanesco[243] obtained impure cultures of *S. gracilis* and *S. balanitidis* by means of gelatinized horse serum. A spiral organism, *S. phagedenis*, was also obtained by me in a pure culture from a phagedenic ulcer on the genitalia of a woman, but its systematic affinity is quite uncertain.[95] *T. calligyrum* is slightly coarser than the pallidum, but is apt to be mistaken for the latter in cultures. It grows easily in tissue-free media.

From dental deposits of normal oral cavity *Treponema macrodentium* and *T. microdentium*[102] were cultivated in the pure state by means of the same methods, and *Treponema mucosum*[103] from the scraping of the pyorrheal gum. The microdentium and the mucosum appear very similar but can be distinguished by the production of a thin but tenacious mucin in the culture of the latter. When the culture gets old, both of these give a strong, somewhat offensive odor. This faculty to decompose the proteins (thus causing a slight turbidity in the fluid media) makes this culture readily distinguishable from the pallidum cultures, because the latter do not produce such an odor. Morphologically, they bear a great resemblance to the pallidum, although their curves are set somewhat more closely than in the pallidum. The macrodentium is more difficult to cultivate and, according to my experience, requires fresh tissue in the culture media. Morphologically, it is coarser than the pallidum and its serpentine movements and irregular, stretchable and wider curves are characteristic enough to distinguish this species from other varieties. It does not produce an odor.

Mühlens and Hartmann[244] succeeded in 1906 in obtaining a pure culture of *S. dentium* in the horse serum agar medium of Mühlens. In their culture they recognized a minute form of Koch's *S. dentium* type and another which approached the dimension of Hoffmann-Prowazek's S. media type. They suggested the possibility of these representing different stages of development or even a sexual differentiation. It appears as though their so-called pure culture may have contained more than one species. No admixture of tiny and coarse forms has been observed in the culture of the macrodentium or microdentium. The dentium culture of Mühlens produced a strong offensive odor.

IMMUNITY AND IMMUNIZATION.

The very name, relapsing fever, suggests the possibility of the development of some sort of protective power in the infected hosts against a third attack. In

fact, the second attack is often milder than the first and a third relapse is rare. Persons who have had the fever are usually immune to subsequent infection for a period of several years. The same is true of the African tick fever although repeated recurrences are more frequent in this instance. Susceptible animals, such as monkeys, mice, and white rats, enjoy a period of immunity extending over about three months after recovering from the second attack. In rats no relapse has been observed. In the fowl and geese, similar immunity follows recovery from spironematosis. The studies of various investigators, especially Gabritschewsky, Pfeiffer, Novy and Knapp, Manteufel, Marchoux and Salimbeni, Levaditi and Manouélian, Prowazek, Neufeld, and others, have contributed in explaining the mechanism upon which the immunity depends. Gabritschewsky[245] demonstrated the presence of a specific antibody against *S. recurrentis* in the blood of convalescent patients by mixing it with the spironema-containing blood *in vitro*. The destruction of the organism occurred within a short time when the mixture was kept at a temperature of 37°C. This author considered that the development of a germicidal substance in a patient's blood was the cause of the crisis and subsequent immunity. He also recognized the appearance of a similar specific immune substance in the geese recovering from the attack of *S. anserina*. The convalescent recurrentis blood had no effect upon the organisms of goose fever and the anserina blood did not affect the organisms of the relapsing fever. The phenomena observed were agglutination, immobilization and dissolution of the organisms when mixed with their correspondingly specific bloods. Gabritschewsky produced an immune serum by injecting the horse with a spironema-containing blood. It was tested by Löwenthal in 83 cases, and in 39 cases (47%) no relapses occurred, while in 140 untreated cases, 65 had three attacks (46.5%). Novy and Knapp[18] confirmed and greatly extended the experimental part of Gabritschewsky's work and pointed out that the protection afforded by active as well as passive immunity is not wholly dependent upon the germicidal property, but also upon the immune bodies, since a comparatively weak germicidal blood may protect the animal against the infection in small quantities. Besides, Novy and Knapp hold the role of the phagocytes (mononuclear, but not polynuclear) to be very important, as they ingest the dead as well as the enfeebled spironemata under the influence of immune bodies. Levaditi and Manouélian[246] suggest the existence of an opsonin in this phenomenon. Manteufel[247] believes the lysis of the spironemata in the immune serum to be due to the cooperation of complement and a specific amboceptor. The rapidity and intensity with which the spironemata are destroyed within the peritoneal cavity of actively or passively immunized rats is variable. In the peritoneal cavity of a hyperimmunized animal the organisms become granular in from two to five minutes, while in rats recently recovered from the attacks, the organisms are ingested in fifteen minutes. In passively immunized rats the spironemata are first agglomerated and temporarily immobilized and this is followed by the appearance of some leucocytes on the scene; but the effects of the immune substances gradually wear off within about an hour. The leucocytes disappear in 30 minutes (Novy and Knapp). The germicidal and bacteriolytic actions are parallel. The duration of passive immunity in rats is less than forty days while that of the active immunity lasts nearly four months. Novy and

Knapp succeeded in preparing in rats by means of hyperimmunization a powerful immune serum which contained in each cubic centimeter 500 immunity units; that is, 0.002 c.c. of the serum was able to protect the rat against 0.1 c.c. of the infective blood showing 10 to 50 spironemata per field (2-mm. objective). In ordinary recovered rats there were only about 2 immunity units per cubic centimeter. The use of the immune blood from a hyperimmunized rat prevented the infection in the rat and cured it on its onset, but a greater amount is found necessary in order to obtain similar results in monkeys and mice as these animals are subject to a relapse after the treatment. Novy and Knapp suggested the inoculation of the Spironema during the apyretic period in order to increase the amount of immune principles in the victim's system and thereby ward off a relapse. They found an interesting phenomenon; i. e., the injection of too much immune blood proved to be less effective than a moderate quantity. This was explained by assuming the production of a specific precipitin which acted as an anticomplement. In regard to the use of hyperimmunized blood serum in human relapsing fever, they calculated that about 375 c.c. of a serum such as mentioned in the experimental part would be necessary and that the future of a serotherapy much depended upon the success attained in cultivating the organism in an artificial medium in large quantities. As a matter of fact we have been able to collect large quantities of comparatively pure organisms from each of the cultures of *S. recurrentis, S. duttoni, S. novyi, S. gallinarum,* etc., for various purposes (immunization, vaccinotherapy, etc.). In the serum of those who had just recovered from the relapsing fever, a complement fixation principle was demonstrated by Kolle and Schatiloff,[248] and Korschun and Leibfried.[249] The reaction was said to be positive after the second attack.

In Weil's disease Inada and his coworkers found the presence of a specific spironemalysin in the serum of convalescent man or guinea pig. The immune bodies develop after the second week of the disease and may be still present in individuals who had the attack more than four years previously. The Pfeiffer phenomenon is easily demonstrated by using the organ (liver or kidney) emulsions rich in the spironemata or a culture and the immune serum in the peritoneal cavity of the guinea pig. These investigators immunized goats and horses with the cultures of the causative agent (*S. icterohemorrhagiæ*) for a period of more than a year and succeeded in producing a serum which prevents the infection against the lethal dose in guinea pig in the amount of about 0.001 c.c. The clinical experience of this serotherapy which has now extended over many hundreds of cases proves to be highly encouraging.*

The question of immunity in syphilis is rather imperfectly understood. In human subjects it was once assumed that after the first infection, complete immunity occurred as evidenced by the extreme rarity of a reinfection. Later investigators seem to consider this assumption as incorrect inasmuch as it was based upon the fact that the syphilitic individuals do not a second time contract a chancre or show a general skin eruption in spite of exposure to such an infection. This fact does not, however, necessarily denote immunity in the usual sense of the word. This state of refraction to the second infection is said to be due to the pre-existence of the same virus in the same individual who no

*Personal communication, soon to appear in print.

longer reacts to the second inoculation with the original intensity or vigor, and the condition is designated by Neisser as "Anergie." At the same time Hutchinson showed the possibility in rare instances of an autoinoculation, while Finger and Landsteiner[250, 251, 252] believe that a superinfection may take place in certain syphilitics. The effect of a superinfection may be a purely local manifestation or it may be subsequently followed by generalization; or it may again cause a general mobilization of the virus without a local manifestation. The character of the lesions produced by superinfection agrees with that of the lesions peculiar to different stages of the disease. If it occurs during the secondary stage, the superinfected lesion will be a papule or other exudative product, and if during the tertiary stage, the result will be a gummatous product. This alteration of various tissues of a syphilitic individual in their reactivity to the syphilitic virus is designated as "Unstimmung" by Neisser who regards this condition as a morbid state of the tissues brought about by the pressure of *Treponema pallidum*. There once prevailed a vague impression that when cutaneous tissues are extensively involved there is less likelihood of the visceral organs being invaded by the syphilitic virus and vice versa,[253, 254] but there is no experimental proof to support this contention. Since the introduction of salvarsan and its derivatives in the treatment of syphilis, the instances of reinfection with typical or sometimes atypical chancres are not so rare, thus indicating that after a cure has been effected the human body reacts in the usual or nearly usual manner.[255] This also points to the absence in such cases of any lasting immunity after the first infection has been eradicated. A thorough investigation is required in order to ascertain whether or not a certain degree of immunity develops in some of the cured cases, thereby affording protection. In some ways the question of immunity in syphilis is comparable to that in protozoan diseases, in which, though latent, no typical infection can be reinduced until the first attack is completely cured, and where no congenital immunity has yet been demonstrated.

Let me now review the situation of the immunity question in experimental syphilis. Metchnikoff and Roux, Neisser and Bruck, and others found that monkeys that have once been infected with *Treponema pallidum* may prove refractory to subsequent inoculation. Metchnikoff[256] thought he succeeded in protecting a monkey against the infection by inoculating it with an attenuated living virus which was no longer able itself to produce typical reactions. That the vaccination against syphilis was not equivalent to that against variola in its fundamental principle was later demonstrated by Neisser and others, who were able to show that the monkeys that had been "vaccinated" with an attenuated virus and which were rendered "immune" to the subsequent inoculation with a fully virulent material were harboring the infection in various localities escaping the usual clinical detections. Thus the emulsion prepared from the bone marrow, spleen, etc., of the "vaccinated" animals was able to infect new susceptible animals. This phenomenon is similar to the state of anergy observed in syphilitic human subjects. Fontana,[257] Uhlenhuth and Weidanz,[258] Bertarelli,[259] Truffi,[260, 261] and others, pointed out that a rabbit which carries syphilitic keratitis in one eye is not refractory or immune to the infection in the other eye. A rabbit, one of whose testicles is infected with *T. pallidum*, offers no greater

resistance in the other which may be infected with the virus at any stage of orchitis preceding that on the opposite side. Tomaszewski[262] thought that a skin infection produced in rabbits in which scrotal lesions had been persisting for about two months was much milder than in normal animals. According to personal observations, a rabbit in which a syphilitic orchitis, or keratitis, or scrotal chancre has been cured either spontaneously or through the administration of salvarsan enjoys no perceptible immunity to syphilis. Truffi repeatedly inoculated rabbits with a fetal liver emulsion containing an abundance of *T. pallidum,* but found no immunity to develop. Uhlenhuth and Mulzer immunized rabbits with the testicular pallidum emulsion without obtaining any decisive result, although in some cases they thought it exerted a beneficial influence upon the syphilitic process. In my personal experience it has been found that the susceptibility of the rabbit to syphilis is decidedly diminished in some animals by immunizing them with *T. pallidum* for several months. With a strain which gave 100 per cent takes in normal rabbits' testicles, only about 60 per cent positive results were obtained in the immunized animals. This tends to show that the lower percentage of positive takes in the immunized rabbits may be due to the destructive influence of the treatment upon the invading pallida. But it was also found that in the immunized rabbits in which the inoculation succeeded, the symptoms were not any milder. In fact, not only were the local reactions just as marked as in the control animals, but there was a tendency to the formation of generalized lesions. In two of the rabbits scrotal lesions developed after the intravenous inoculation of a virulent strain. It appears that an incomplete immunization exerts an adverse influence on the defensive factors of the rabbit. This phenomenon finds verification in the work of Grouven and Sowade,[263, 264] who recommended for the animal a few preliminary intravenous inoculations of the pallidum in order to insure a generalized infection through a subsequent intracardial introduction of the organisms in huge quantity. I also endeavored to ascertain whether a local administration of devitalized pallida (killed at 60° C.) on many successive occasions will not bring about a state of local immunity to *Treponema pallidum,* but my results were rather unsatisfactory, for the reason that the testicular parenchyma which was repeatedly inoculated with the pallidum emulsion underwent gradual atrophy, and the resulting hard fibrous structure was no longer a suitable test object for this fastidious parasite. Nevertheless I was able to produce small nodular lesions in two out of several rabbits so treated. Moreover, reinfection of the same tissues (cornea, testis, and skin) after a spontaneous or chemotherapeutic healing has been found possible as long as the suitable structures of the tissues are preserved.

Our knowledge pertaining to the immunity phenomena *in vitro* is of more recent date, for the test tube experiments with *T. pallidum* were made possible since the discovery of the organism and were particularly favored by the successful cultivation of the parasites on artificial media. Attempts to demonstrate the presence of a specific agglutinin for *T. pallidum* in the sera of human and experimental syphilis were made by Hoffmann and Prowazek,[265] Herxheimer and Löser,[266] Hoffmann,[267] Brönnum and Ellermann,[268] Babes and Panea,[269] Metchnikoff and Roux, Landsteiner and Mucha,[270] Zabolotny and Maslakowetz,[271]

and others with the pallida derived from the syphilitic tissues. Their experiments were indecisive, owing to the difficulty found in obtaining a pure material free from various tissue constituents. Uhlenhuth and Mulzer[189] found no agglutinins to be formed in the sera of the rabbit, goat and monkey after repeated intravenous injections of the rabbit's testicular emulsion rich in the pallidum. In 1910-1911, soon after obtaining pure cultures of *T. pallidum*, we started the immunization of rabbits with different strains of the organism. In the sera obtained from the immunized rabbits we were able to demonstrate the presence of the specific agglutinins and complement-binding principles for the cultivated pallidum strains. We were unable to produce with the sera any unmistakable agglutination of the pallidum derived directly from the syphilitic orchitis of the rabbit, but I considered this to be due to the simultaneous presence of tissue debris and other cellular elements which may have interfered with the agglutination phenomenon. These sera were not strictly specific, but contained a small quantity of agglutinins for other treponemata obtained in pure cultures. There were also a sufficient number of specific complement-binding bodies, but there was at the same time a more or less definite group reaction for other treponemata. The work was continued later by Akatsu at my laboratory with similar results. He was able to obtain a serum which could agglutinate the pallida in a dilution of 1 :50,000.

In order to know whether syphilitic human sera have any definite agglutinating and complement-binding properties, a number of sera obtained from various stages of syphilis were examined with pure cultures as well as with the tissue pallidum derived from rabbit testicles. All the experiments were unsatisfactory, owing to the difficulty experienced in reading the reaction in the case of agglutination and also owing to the high anticomplementary powers of the antigens and the feebleness of the reaction in the case of the complement fixation test, except in the case of the pure culture antigens which fixed complement with the immune rabbit as well as with some of the syphilitic human sera (chiefly late and tertiary cases). According to our experiments, there is a certain degree of group reaction for the other treponemata (*T. calligyrum*, *T. microdentium*, *T. mucosum*, and *S. refringens*).

Kolmer[272] first described the agglutination of a pure culture of *Treponema pallidum* by the sera of rabbits injected with a living and heat-killed culture furnished by our laboratory. His results show that normal rabbit sera do not agglutinate the culture pallidum in dilutions as low as 1 :20, while the sera of immunized animals produced agglutination in dilutions as high as 1 :1,280. No definite agglutination was observed with human syphilitic sera in a dilution of 1 :20 or higher. Nakano[273] also reported the presence of agglutinins in the sera of rabbits injected intravenously with a pure culture in dilutions from 1 :10 to 1 :70. Kissmeyer[274] immunized rabbits with a pure culture of *T. pallidum* and was able to obtain agglutinations in dilutions as high as 1 :200,000 to 1 :500,000 of the immune sera, while the sera from individuals with primary, secondary, tertiary and congenital syphilis contained agglutinins for the pallidum in dilutions of 1 :100 and higher in a percentage of from 40 to 60 out of 59 cases. Normal human sera may agglutinate the pallidum in dilutions as high as 1 :50. Zinsser and Hop-

kins[275] state that normal rabbit serum may agglutinate the pallidum in dilutions lower than 1:10, but the sera of their immunized rabbits (intravenous injections of the pallidum cultures) agglutinated it in dilutions as high as 1:2,000. They added that the normal as well as certain syphilitic human sera may agglutinate the culture pallidum in emulsions. Zinsser, Hopkins, and McBurney[276] failed to observe any agglutination when the pallida from human lesions were mixed with the immune sera (rabbits and sheep) produced with the culture pallida. Zinsser and Hopkins[277] demonstrated the treponemicidal bodies for *T. pallidum* (cultivated) in the immune serum produced by them.

In the sera of animals experimentally infected with syphilis the presence of specific complement-binding antibodies for *T. pallidum* has not been satisfactorily proved. It is true that we were able to demonstrate the positive complement fixation in the sera of animals immunized with cultivated treponemata, but this does not hold good when dealing with the syphilitic animal sera and the virulent pallidum strains found in tissues. On the other hand, these syphilitic sera do bind complement when mixed with pure cultures, not only of *T. pallidum*, but also of various bacteria, such as colon bacilli (Zinsser and Hopkins). Undoubtedly the phenomenon is nonspecific but pathognomonic as is the Wassermann reaction which is caused by certain lipoidal substances. These cultures must serve as the containers of the similar lipoids. Indeed, Craig and Nichols[278] long ago showed that the alcoholic extracts of the pure pallidum and pertenue cultures produced almost equally strong complement fixation when mixed with the human syphilitic sera, giving a positive Wassermann reaction with pure lipoidal antigens derived from other tissues. In a word, a syphilitic animal may give a positive complement fixation with various lipoids without at the same time containing any specific antibody for *T. pallidum*. In human syphilitic sera the same is also true, except in the sera of certain late and tertiary cases in which there may be a positive reaction due to the specific antigens and antibodies in the strict sense of Bordet-Gengou's phenomenon.[279]

The nature of the Wassermann reaction in the sera of human and experimental syphilitic subjects is still unexplained, but one fact has been established; viz., that it is due to a peculiar change of the sera not specific for syphilis; it occurs in yaws, leprosy, trypanosomiasis, malaria (febrile period), and sometimes in malignant tumors. The fact that so many lipoidal substances as well as certain salts (sodium taurocholate, sodium cholate, etc.) derived from different sources can bring about a positive fixation precludes any strict specific antigen-antibody reaction. According to personal observations the lipotropic complement fixation reaction is not present in immune rabbit sera which have been obtained by injecting the pallida repeatedly, and which contain a large number of specific complement fixation bodies from the pallidum strains employed for their production.

Closely related to immunity is the question of allergy in syphilis. From the chronic nature of the disease many investigators considered the possibility of its occurrence at one stage or another. Jadassohn, Meirowsky, Ciuffe, Fontana, Neisser and Bruck, and others made numerous observations which rendered the presence of allergy still more probable. These investigators were

handicapped by not having a pure culture of *T. pallidum*. Soon after the isolation of the pallidum strains, study of this subject was made in human syphilis with the pure material. In the meanwhile it was ascertained experimentally that the prolonged treatment of rabbits with intravenous injections of the pure pallidum culture as well as with the organisms obtained direct from the rabbit orchitis lead to the production of a state of hypersensitiveness of the skin to the inoculation of the extract of a pure, heat-killed pallidum culture.[280] The reaction was found to be apparently specific for *T. pallidum*. There was no injurious effect following the injection into the rabbits of the heat-killed pallidum emulsion. The emulsion, since known as luetin, was employed as a means of diagnosing human syphilitic cases, with the result that the luetin reaction was found to be most frequently present in the latent, tertiary, and congenital syphilis cases where one would naturally expect most constantly to find the allergetic or hypersensitive state of the skin. As an auxiliary or supplementary factor in producing a positive luetin reaction, I have already pointed out that the pathological state of the skin of chronic syphilitic patients designated by Neisser as "Umstimmung" played a role in nearly 10 per cent of tertiary cases in which the skin reacted intensely to the inoculation of the control emulsion without the pallida. No efforts were made to explain this peculiarity of hypersensitiveness of the skin of certain syphilitics. But recent work of Camp[281] points out that the administration for many days of potassium iodide to a nonsyphilitic individual produces in the skin a hypersensitiveness to any trauma, including the inoculation of the luetin. Probably this finding may furnish the solution of the problem of Neisser's "Umstimmung," or at least of one of the contributing factors. The clinical evidence thus far accumulated seems to show, however, that in a large number of cases the luetin reaction was positive in spite of the fact that no iodide had been given during the period when the test was applied. Recently Akatsu,[282] at my laboratory, carried out several series of experiments regarding the influence of potassium iodide upon the reactivity of the skin of rabbits to the intradermal inoculation of the luetin, control fluid, and plain bouillon. The iodide was administered intravenously for a period of from 7 to 9 days, given in increasing doses of 0.5 to 2 c.c. of a 10 per cent aqueous solution. At the end of seven days or later the skin was tested for the luetin, control, and plain bouillon. It was found that the skin of normal rabbits did not react to the injections after the iodide treatment. There was no change in its reaction to the trauma. The skin of the rabbits which had been previously rendered hypersensitive to the luetin by means of prolonged immunization with pure pallidum cultures mostly remained the same, that is, it reacted to the luetin with the same intensity as it did before the administration of potassium iodide. Only in a few instances was the reaction somewhat intensified. There was no definite reaction to the control emulsion of plain bouillon. In some rabbits, in which the testicular orchitis after several months had shrunk to a small fibrous nodule, the luetin reaction was mildly positive, but the intensity of the reaction was but little influenced after the injection of potassium iodide, except in a few rabbits where the second tests came out more distinctly. The above findings show that the potassium iodide has no noticeable influence upon the reactivity of the skin of normal as well as of syphilitic rabbits. It would be interesting to

study whether in other spironematoses (relapsing fever, tick fever, rat-bite disease, and infectious jaundice) there appears any skin allergy comparable to that described for other bacterial infections (typhoid, gonorrhea, etc.). In cases of yaws, the skin reacts to the intradermal inoculations of the luetin and of the framboesin with equal intensity and cannot be differentiated by this method (Baermann and Heinemann).[283]

The last and probably the most important field for medicine is chemotherapy. The inauguration of modern chemotherapy by Ehrlich is as interesting as it is romantic. It can be traced back to Schaudinn's suggestive but unsupported theory that the spirochætes represent a stage of the life cycle of trypanosomes, or at least were closely related to the latter. The introduction of organic compounds of arsenic into the treatment of trypanosomiasis was promising much when Schaudinn discovered *T. pallidum*, which he regarded as a protozoon allied to the trypanosomes. Ehrlich took up experimental chemotherapy in connection not only with the latter, but also with the newly discovered spirilloses, as he called them, including syphilis and the fowl fever caused by *S. gallinarum*. The achievement of Ehrlich and his collaborator Hata, in discovering salvarsan for the treatment of these two diseases, marks a new era in modern chemotherapy. To review this phase of the spirochæte problem would be out of the scope of my present paper. Suffice it to say that to the great pioneers, Schaudinn, Ehrlich, Metchnikoff, and Neisser, we owe an inestimable debt, not merely for their own researches, but also for re-kindling in us the sublime stimuli which have already inspired so many investigators to discover new facts, and which will continue to urge us still more to take up this task and to extend our knowledge regarding the classification, morphology, biology, pathogenesis, and experimental as well as clinical aspects of the microorganisms known as spirochætes.

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THE EFFECT OF EXTENSIVE VENESECTION AND TRANSFUSION ON KIDNEY LESIONS IN SEVERE ACUTE MERCURIC CHLORIDE POISONING*

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THE manner in which the destructive changes found in kidney, colon, and liver are induced in mercuric chloride poisoning is at this date still a subject of controversy. Whatever the mechanism, it is true that death in man, in a large majority of cases, results from the destructive action, direct or indirect, on renal parenchyma. McNally and I[1] have shown that irreparable kidney parenchyma degenerations (as far as the life of individual renal cells is concerned) can be produced within 3 minutes after the administration per os of a massive toxic dose of mercuric chloride. Chemically the mercury can be detected and is present in the circulating blood in amounts that can be determined quantitatively within a short period of time. Renal cells, like other tissue cells, die and are replaced many times in conditions of normal health or in disease. Even in sublimate poisoning, heroic attempts at extensive regeneration and substitution of the destroyed renal cells are made by the kidney as has been shown by Heineke[2] and many others. When, however, the destruction of the renal epithelium involves tubules practically en masse, and when the remaining undestroyed excretory cells have undergone such severe degeneration that they are unable to functionate, the individual is usually doomed to death from the resulting uremia before new cells capable of functioning can regenerate, or before the injured cells can rehabilitate themselves sufficiently to again take up their own and the added work of their destroyed neighbors.

Modern therapy in the treatment of acute mercurial poisoning has concerned itself principally with attempts at the removal of the unabsorbed poison from the gastrointestinal tract. Lieb and Goodwin[3] have shown that mercury, after its absorption, is excreted into the stomach only to be reabsorbed unless removed by aspiration. Flexner and Sweet[4] have shown that mercury absorbed from the gastrointestinal tract is in part excreted by the liver into the bile which conveys it to the small intestine where it is again absorbed and taken back to the liver. Thus the brunt of the final elimination is borne by such organs as the kidney and colon. It is because of this repeated reabsorption by some organs that the complete elimination of the mercury is such a long extended process. Clinicians have profited by past experimental work, and at present, frequently repeated gastric lavage and colonic flushing or continuous irrigation are yielding most gratifying results as evidenced by the work of Lambert and Patterson[5] and many others. Despite the fact that the lives of some individuals are saved in this way, the prognosis in many cases must still be considered dubious. The fact that, within a few moments after the ingestion of a massive toxic dose of mercuric chloride, sufficient mercury has been taken into the circulating blood to produce most extensive kidney degeneration would indicate that, in conjunction with our present

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therapeutic measures, an additional therapeutic method which would aid in the removal of the mercury already absorbed and present in the blood would be of inestimable value. Its value would be proportionately enhanced if this removal could be accomplished speedily and through some other portal than an excretory organ. As has been indicated the vicious circle established by the continued reabsorption following elimination by other organs increases and prolongs the task set for the kidney. Rosenbloom,[6] in extensive separate analyses made of the organs and body of a girl suicide who died ten days after taking 15 grams of mercuric chloride, found that there was still present in the entire body 175.39 mg. of mercury. Almost 1/3 of this amount, 52 mg., was contained in the blood alone. Almkvist[7] has found experimentally the greater bulk of mercury contained in the animal body to be present in the blood, lymph, and "tissue juices." If then one could remove from the blood all of the absorbed mercury in addition to preventing further absorption of the mercury at its portal of entrance (most frequently the stomach), the kidney and other vital organs would be spared the further onslaught of the toxic agent contained in this blood and they would recuperate more rapidly. That this thought has occurred to many workers is evidenced by a large volume of experimentation recounted in the earlier literature. In most instances it was attempted to render the mercury contained in the blood inert by changing it to an insoluble compound. Various chemicals have been injected intravenously or administered by mouth for this purpose. Results have been variously reported. Therapeutically all of these methods have been abandoned by most clinicians who have attempted them. If, as it would seem, it is impossible to remove mercury satisfactorily in this way, one other method, to date apparently untried, still remains open to us. Recent successes and simplifications in the methods of transfusion would seem to indicate the way to this last avenue of approach—complete substitution of the mercury-containing blood by nonmercury-containing blood. Alternating copious venesection followed by transfusion of normal blood would assuredly aid in removing the toxic substance. Whether the degree to which the kidney could be spared would warrant such a procedure seems on the surface problematical, even doubtful. Viewed in its most favorable light, little could be hoped for unless one were certain that there would be no further absorption of mercury to contaminate the newly transfused blood. At best, then, this procedure could be only a supplementary therapeutic aid. Incidental factors such as the initial damage done to the kidney and the completeness and rapidity of the removal of the circulating mercury must assuredly be of much moment in a problem of this type. With an hypothesis of this character, admittedly of dubious prospect, yet not without its ray of promise, the following experiments were undertaken:

A series of 14 dogs and 24 rabbits were used. One-half of this number were used as controls. For each animal transfused a separate control of approximately the same size, weight, and sex, was chosen. Where there was a difference in weight, the larger animal was chosen as the control. Young dogs weighing from 4 to 7 kilos were used. In our previous work McNally and I[1] found that mercury is absorbed and taken into the blood very rapidly when administered by mouth to an empty stomach, and that practically simultaneously tissue changes in remote organs appear. It was therefore decided to admin-

ister the mercuric chloride to these animals subcutaneously. This assured absorption as rapid as that from an empty stomach. In addition, complete anesthesia to prevent loss of mercuric chloride by vomiting could be eliminated. Mercuric chloride in a slightly acid water solution (15 to 20 c.c.) was administered in amounts varying from 0.25 to 0.5 grams. This was injected subcutaneously in amounts from 2 to 5 c.c. in various parts of the body thus promoting most rapid absorption. The injections in the controls were in the same amounts and in corresponding subcutaneous locations. Blood corpuscles which had been previously obtained from normal dogs' blood, had been washed free from their plasma and had been kept in a solution of 2½ per cent dextrose in Ringer's solution at 4° C.,[8] were used in the transfusion. The blood treated in this way was obtained from 1 to 3 days before it was transfused. The animal was transfused from 2 to 3 hours after the administration of the mercuric chloride. No preliminary examination was made to determine the presence of isoagglutinins or isohemolysins on the part of donor or recipient. The animal was transfused under ether anesthesia. Bleeding and transfusion was done by means of a 100 c.c. Luer syringe and glass cannula, via the right jugular vein. From 100 c.c. to 200 c.c. of blood were withdrawn at one time and followed immediately by slow indirect transfusion of an equal volume of the washed corpuscle suspension. This was continued until 400 to 700 c.c. of blood had been removed. In the smaller dogs the ultimate volume removed in this manner was equal to 1½ times the total normal blood volume for the animal. In the larger dogs this represented usually slightly less than the total amount of blood. From 1½ to 2 hours were usually occupied in making this blood substitution. The vein was tied and the skin was closed. The control animal received no treatment. Death invariably occurred within 18 to 24 hours. With one exception all of the transfused animals died 2 to 8 hours earlier than the controls. In the former instance the death of the control occurred 8 hours prior to that of the transfused animal. In the rabbit series blood from normal rabbits was obtained and washed as was the dogs' blood. Rabbits weighing from 1500 gm. to 2500 gm. and equally heavy control animals were used. Mercuric chloride in amounts of 0.01 gm. to 0.06 gm. was injected subcutaneously similar to the manner in which it was administered to the dogs. Because of the difficulty experienced in obtaining generous amounts of blood by venesection, the rabbits were bled by cardiac puncture. They were then transfused through a marginal ear vein with a suspension of washed corpuscles from an equal volume of normal rabbits' blood. In five rabbits and their five controls death occurred within 2 to 4 days. Three of the transfused animals lived from 10 to 20 hours longer than their controls; the remaining 2 were found dead in their cages 4 to 8 hours before the death of their controls occurred. Three rabbits died within a few hours following cardiac puncture and transfusion. At necropsy death was found to be due to a heart tamponade secondary to intrapericardial hemorrhage from the cardiac puncture wound.

A comparative study of the kidneys of these animals and their controls which seemed to warrant a continuance of the work will be discussed later. Had the mortality rate in these experiments been less discouraging there would still have been a most serious drawback to the practical application of this method of therapy, i. e., the difficulty experienced in obtaining donors for such large quan-

tities of blood. I therefore decided to attempt a removal in vitro of the mercury from the blood obtained by venesection, and to utilize this blood in a subsequent transfusion. In medico-legal investigations the presence of mercury is frequently determined quantitatively by electrolytic methods. A modification of the Wolff-Schneider[9] apparatus for this purpose was kindly designed for me by W. D. McNally. This consisted, as will be noted from the accompanying illustration, of a glass container for the blood. In this were immersed a funnel-shaped gold, and a spiral-shaped platinum electrode. Two dry cells in series, or a 4 volt storage battery were used. The platinum electrode was connected to the cathode, and the gold to the anode. In a series of preliminary experiments, blood from dogs poisoned with mercuric chloride was washed by the method previously cited. Fifty to 70 c.c. of such washed corpuscles when tested for mercury always gave a most marked positive Reinsch test. An equal quantity

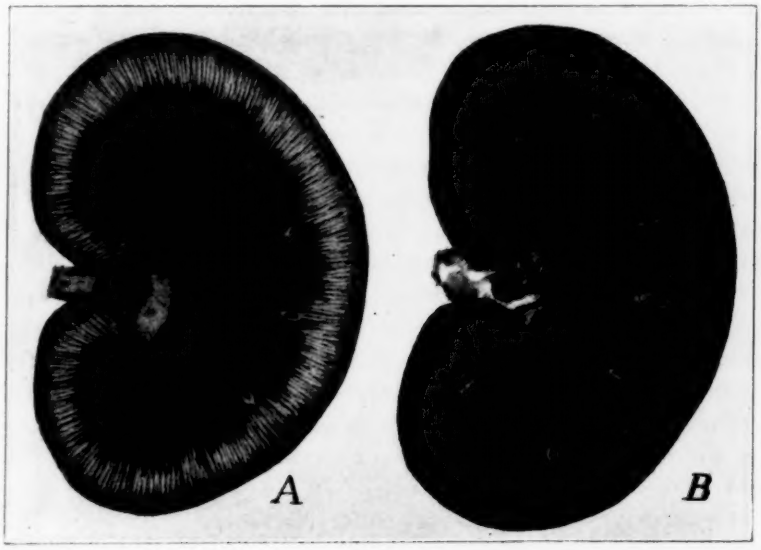

Fig. 1.—*A*. Kidney of control dog which received no treatment. Grossly the changes of fatty degeneration located in the epithelium of the ascending limbs of the loops of Henle are very evident. *B*. Kidney of dog treated by venesection and transfusion. This dog was approximately of the same age, of equal weight, and the same sex as the dog from which *A* was taken. He received a dose of mercuric chloride subcutaneously equal to that of *A*.

of such blood placed in this apparatus was negative for mercury by the Reinsch test after a current had been passed through it for 45 minutes. In careful hands it has been found that the Reinsch test will yield positive results where as little as 0.00001 gm. of mercuric chloride is present in 100 c.c. of suspected material. During this mercury-removal process bubbles of chlorine and other gases were liberated and rose to the upper surface carrying along some clumps of erythrocytes enmeshed in a small amount of fibrin-like material. After 45 minutes the contents of the reservoir were drawn off and filtered under sterile precautions through 8 to 10 layers of surgical gauze. When examined microscopically, the erythrocytes appeared decidedly crenated. There were no blood shadows, no fragments of corpuscles, and the corpuscles were not clumped. It was found, however, that from 30% to 50% of the corpuscles were lost during these manipu-

lations. Because of this the corpuscles were again washed and recentrifugalized. By withdrawing a sufficient quantity of the upper clear fluid after centrifugalization, the total volume of the corpuscle suspension was reduced until counts yielding from 7,000,000 to 8,000,000 erythrocytes per c.mm. were obtained. Three rabbits poisoned with mercuric chloride were exsanguinated and their blood treated in this way. Three normal rabbits weighing from 1400 gm. to 1500 gm. were then bled by cardiac puncture and 50 c.c. to 60 c.c. of blood was removed from each. These rabbits were immediately transfused through a marginal ear vein with an equal volume of the corpuscle suspension obtained from the blood of the poisoned rabbits. There were no untoward results. The rabbits, in collapse following the cardiac puncture, were on their feet within half an hour and during the following 20 days were apparently normal. They were subsequently used in other work. To each of three rabbits weighing 1500 gm. to 1600 gm. was then administered 0.01 gm. of mercuric chloride hypodermatically. After one hour they were bled by cardiac puncture to the extent of 50 c.c. of blood and immediately transfused through a marginal ear vein with an equal quantity of blood from normal rabbits. The blood removed by cardiac puncture from each rabbit was washed and freed of its mercury by the method described; the volume was adjusted to an 8,000,000 per c.mm. erythrocyte count. Enough normal rabbits' blood was added to each portion to bring the volume to 40 c.c. One to two hours following the first cardiac puncture the animals were again and similarly bled to the extent of 40 c.c. of blood and immediately transfused as before, this time, each with its own blood. This was the blood which had been obtained at the previous bleeding and freed of its mercury in the interim. Death in all three instances ensued within the next hour. Apparently the shock attendant upon two cardiac punctures was too great. The three control animals died within two to three days.

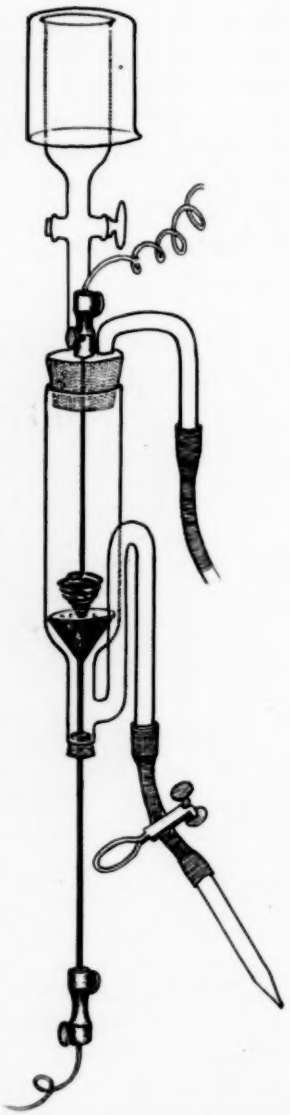

Fig. 2.—Apparatus for removing mercury electrolytically from the blood.

At necropsy in the rabbit series no striking differences were noted grossly in a comparison of the kidneys of the control and of those of the transfused animals. In the dog kidneys, however, relatively marked differences were apparent. The light ray-like streaks of fatty degeneration (microscopically usually limited to the ascending limbs of the loop of Henle) so characteristic of acute mercuric chloride poisoning in the dog, were in all instances less numerous and decidedly fainter in the transfused animals than in their controls. This was equally striking

microscopically in Sudan III stained sections. In most instances fewer ascending limb tubules—numerically ⅓ to ½—were involved, and the degree of fatty degeneration of the epithelium was far less advanced. Frequently only a few cells in the entire tubule presented these changes, whereas in the control animal every cell in an entire tubule was found involved, and this was practically uniform for all tubules of this type. No material differences, however, were found in the degree of degenerative change in the convoluted tubule epithelium. In both transfused and control animals severe parenchymatous degenerations were usually present in these tubules. The minute black globules and granules described by Almkvist[7] and others which are to be seen in parenchyma cells and blood and lymph channels in the kidney of mercuric chloride poisoned animals, were quite as abundant in the kidney of the transfused as in the nontreated animals.

CONCLUSIONS.

1. Copious venesection followed by transfusion of normal blood inhibits both qualitatively and quantitatively the characteristic degeneration usually found in the epithelium of the ascending loop of Henle in the kidneys of dogs acutely poisoned by mercuric chloride.

2. In desperate and other cases of acute mercurial poisoning, venesection followed by transfusion should be practiced in addition to other therapeutic measures now in use.

3. Erythrocytes, from the blood of rabbits, when suspended in Ringer's solution are apparently not injured by a 3 to 4 volt electrical current passed through the solution for ½ to ¾ hour periods.

4. In this way metallic mercury can be removed in vitro from the blood of animals poisoned by mercuric chloride and this blood qualitatively freed from its corrosive contamination can again be utilized in subsequent transfusions.

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RECENT ADVANCES IN THE STUDY OF AUTOLYSIS*

By Max Morse, Ph.D., Chicago.

THE purpose of this brief communication is to present some of the more recent work concerning the behavior of intracellular enzymes, which is of more definite interest to the clinician. An exhaustive account of modern investigations in this field would involve lines of work, which, while of the greatest importance in themselves, are of lesser importance to the medical man. As an example, we may mention yeast autolysis, where the problem of hydrolysis and synthesis is being considered. Meager reports have been made concerning the role of yeast proteolytic enzymes in synthesizing proteins for human food; but interesting as these matters are, we shall confine the following pages to a more circumscribed field. For general considerations, the reader is referred to the excellent summary of Wells,[1] to the book of Carl Oppenheimer,[2] and to Vernon's article.[3]

It is widely accepted as a matter of demonstrated fact that autolysis; that is, the process of functionating of intercellular enzymes, plays a major role in pathology. Thus we find the statement made that "all absorption of dead or injured tissues seems to be accomplished by means of digestion by the enzymes of the cells and tissue fluids,"[1] The removal of infarcted tissues, digestion of necrotic areas, involution of the uterus, liquefaction of nervous tissue, dissolution of pus, disappearance of pneumonic exudates, atrophy of the liver, and many other pathological phenomena are assigned to autolysis in causal relations.

Upon what basis do we rest our belief that intracellular enzymes are responsible for these processes? The evidence is *a posteriori* in each case. Ligate an organ from its blood supply and estimate the amino-acid content of the organ; amino-nitrogen has markedly increased.[4] Study newly formed pus and compare it with that which has existed for some time; amino-acid increase again in the latter case.[5] Excise an organ, say, the spleen. Grind it up, suspend it in water, and cover with chloroform and toluene, permit to stand a few days in a temperature of 37° C; dissolution and an increase, again, of amino-acids.[6] The evidence, then, is that inasmuch as there is an appearance of the end products of erepsin-like digestion, erepsin-like digestion has taken place.

However willing we may be to accept this analysis as final, we must answer the following questions before we may legitimately relegate the processes enumerated above to autolysis, as the term is defined:

1. Why is it that there is a wide discrepancy in rate between *in vitro* autolysis of brain, muscle, etc., and the instances referred to autolysis involving these tissues, mentioned above? The liquefaction of brain tissue is remarkable for its rapidity, yet brain tissue is the slowest in rate to autolyze[4] out of the body. The same statements may be applied to muscle, except that the discrepancy is less marked.

*From the Nelson Morris Memorial Institute for Medical Research of the Michael Reese Hospital. Presented at the January, 1917, meeting of the Clinical Society of the Hospital.

2. Why is it that an actual acidity (pH < 7.07) is necessary for *in vitro* autolysis, [7, 8, 9], while in no instance has a true acid reaction been attained in the organism save in the stomach and in the urinary bladder?[10] Certainly we cannot align uterine involution with autolysis, for the blood supply is intact and any tendency toward pH < 7.07 is met by buffer salts, as Henderson[10] has shown. We may say the same of certain types of carcinomata, of hemorrhagic infarcts where total occlusion of the blood has not occurred, etc.

Until these discrepancies are explained, we must apply the Scots' verdict of not proved.

Modern studies of autolysis have approached more and more closely to the factors fundamental to the process. Up until the present time, for instance, it has been believed that the effect of acid, long known to accelerate *in vitro* autolysis, was directly upon the enzymes, activating them as we believe pepsin is activated by hydrochloric acid,[11] and as other enzymes are affected by reaction of medium. Bradley,[12] however, has shown that the effect of adding acids or "acid" salts concerns not only the *rate* but *the point of equilibrium of the reaction;* that is, not only is the rate accelerated, but the total quantity of end products of hydrolysis is greater than in the control. Now, Ostwald has demonstrated that a true catalyzer does not affect the point of equilibrium, but the rate alone, and for this reason Bradley has been led to examine the matter further. He has found that the addition of foreign proteins to an autolyzing digest gives the same picture as a digest where acid has been added. He likewise finds that when acid is added to such a digest, the rate and the point of equilibrium are not affected, meaning, as he believes, that no enzymic effect is obtained. Other reasons are given in the original papers, to which the reader is referred. The matter is important because there is no essential difference between the natural accumulation of acids in dying tissues (hydroxy acids, ketone acids, etc.) and the addition of acid from without, artificially. Bradley has attempted to formulate an explanation of starvation metabolism, acidosis, and other pathological states in terms of intracellular enzymes. To this theory the writer has objected as far as interpretation is concerned.[13, 14]

Another theory concerning autolysis is that which has been erected to explain the acceleration at the inception of autolysis where oxygen supply is eliminated. W. E. and E. L. Birge,[15] on studying the effects of oxygen upon pepsin and trypsin, found that the action of these enzymes was suppressed in an atmosphere of oxygen gas and purely as a matter of analogy, they assumed that autolyzing enzymes reacted similarly. That there is no basis for such an assumption has been shown by the writer,[16] who studied the effects of oxygen gas, oxides and peroxides upon autolysis. If oxygen or other oxygen-bearing reagents affect intracellular enzymes in any way, it is an acceleration rather than a retardation, so that any theory based upon the belief that oxygen, when present, as in normal tissues, inhibited the action of these enzymes, while, on interfering with the blood supply, oxygen supply is interrupted and the inhibitory mechanism removed, is untenable. From the time of Hoppe Seyler and his pupil Lubavin,[17] who first recognized autolysis as a ferment action, this erroneous theory of oxygen relation has been held.

What really happens when the blood supply is cut off from an organ is the accumulation of H_2CO_3 and perhaps of acids of incomplete combustion, produced intramolecularly, such as hydroxy acids (lactic, etc.), ketone acids, etc., so that autolysis proceeds in an acid medium.

Regarding the factors operating to permit autolysis in some tissues at a much greater rate than in others, the writer has eliminated two. In the first place, inasmuch as the number of nuclei per cubic millimeter in gland tissue is much greater than the number in tissues such as muscle, nervous, etc., which autolyze more slowly, it is a tempting hypothesis that this factor of nuclear components may be a potent one. To solve at least one portion of the problem, the writer[16] added nucleic acid salts, such as sodium nucleate to autolyzing tissue and determined that there is no acceleration in the presence of the nuclear compounds. There remains, of course the possibility that the enzyme component of the tissues autolyzing at faster rate is greater than that of the more slowly digesting material and the writer is at present attempting to determine this point.

Again, it is possible that some of the tissues which undergo autolysis at greater rate produce more acid in a given time than those autolyzing more slowly. The writer has studied this in a preliminary way, but to an extent sufficient to warrent the statement that as far as brain compared with liver is concerned there is no basis for the assumption. Using the Sörensen colorimetric method with Hynson-Wescott-Dunning tubes with phenolsulphonphthalein as indicator, the tissues were dialyzed within 5 seconds after their excision from the rabbit which had been killed by an occipital blow, against 0.8% NaCl, pH=7.0. The results in both instances ran parallel and approximated, essentially, the findings reported before.[8] At the end of an hour, the reaction gave approximately the same in both cases, pH = 6.4. Inasmuch as the rate of autolysis during this time is a function of the concentration of hydrogen-ions to at least a considerable extent, it seems unlikely that acid development affects the quantitative results in a differential way among the tissues.

It must be admitted, then, that much remains to be accomplished in the study of the fundamental factors in autolysis before we are at liberty to assign limitations of the participations of this process in pathology.

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PERNICIOUS VOMITING WITHOUT ACIDOSIS ASSOCIATED WITH A DISTURBANCE OF THE AMMONIA-UREA QUOTIENT OF THE BLOOD*

By H. L. McNeil, M.D., and M. D. Levy, M.D.,
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SEVERAL forms of persistent vomiting, not connected with any definite demonstrable pathological lesions have been described at different times. Many of these attacks have been attributed to a condition of acidosis, as for example, the cyclic vomiting of children. Others have been explained by pregnancy, pelvic disorders in women, and still others, the so-called idiopathic vomiting of adults, for example, remain unexplained.

It has been the privilege of one of us, during the past few years to have observed two cases of pernicious vomiting in adults, not associated with any demonstrable lesions of the body which could account for the condition, nor with any acidosis so far as was determined. Since these patients presented practically identical clinical pictures, and since one of them came to autopsy, while the other was made the subject of a limited metabolic study by means of some of the newer methods recently placed at the disposal of the clinician, we feel that these cases, taken together, may be worthy of report.

The symptoms of both of these patients were practically identical, consisting in each case, primarily, of severe and persistent periodic vomiting associated with a certain degree of mental unrest, irritability, and towards the termination of the disease, of stupor. Delirium was not noted. No fever, chills, jaundice, nor abdominal tenderness or rigidity was noted in either case. Death terminated the disease in one case, while the other recovered after a serious and prolonged illness.

The cases in detail are as follows:

F. S., 39 years of age, single, a seamstress by trade. Entered the hospital complaining of vomiting which had come on suddenly without apparent cause. Family history negative. Past history negative, except for the continuous use of alcohol in the form of both whiskey and beer for about ten years. The present illness had come on three days before admission, apparently without any exciting cause.

Physical examination on admission revealed nothing of note. No abdominal tenderness or rigidity was noted. Liver apparently not enlarged. Patient was rather dull and stupid mentally, but became irritable and querulous when aroused. Gastric analysis showed an absence of free HCl with an average total acidity of 10. No other abnormalities. Duodenal contents and stools repeatedly negative. Blood showed a slight relative anemia (red blood cells, 4,000,000) and a white count of 9,000 with a normal differential count. Wassermann negative. Urine showed a specific gravity of from 1015 to 1030. Reaction *faintly* acid to litmus. No albumin, casts, or acetone bodies upon repeated examina-

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tions. Death occurred two weeks after admission, the patient gradually becoming weaker, and the vomiting continuing at intervals throughout the course of the disease. Death was apparently caused by gradual cardiac failure. No delirium noted at any time.

Autopsy showed absolutely nothing to account for death, a rather marked degree of cloudy swelling of the liver being the only gross abnormality noted. Microscopic sections of brain, kidneys, pancreas, and liver failed to add any information as to the cause of death.

The second case, seen only recently, who has apparently recovered, is that of a man, white, 60 years of age, a railroad conductor by occupation. Entered the hospital complaining of persistent vomiting. Family history negative. Past history negative except that like the preceding case, he has been a steady drinker for a long period of years, averaging according to his statement, from four to five drinks of whiskey per day for forty years. The present illness began one week before admission with an attack of faintness and dizziness which was so severe as to cause him to fall to the floor (but not to lose consciousness). Subsequent to this attack, periodic vomiting made its appearance and has persisted ever since, coming on at irregular intervals. According to the patient, he has to lie on his right side constantly in order to prevent vomiting.

Physical examination shows a fairly well nourished individual. Heart and lungs negative; peripheral arteries sclerosed; liver apparently not enlarged. No abdominal tenderness or rigidity. Systolic pressure 150; diastolic 105. Mentally, the patient was rather irritable and querulous, although his reasoning power seemed to be fairly normal. Pulse rapid on admission, averaging 110. No fever noted at any time. *Gastric contents* showed an absence of free hydrochloric acid on several occasions with an average total acidity of eighteen. No other abnormalities. Duodenal contents and stools negative. Numerous *urine* examinations showed a specific gravity of from 1010 to 1025, no casts, albumin, or acetone bodies at any time. Twenty-four hour output averaged from 1,000 to 1,200 c.c. Phenolphthalein excretion 55 per cent in two hours. *Blood examination* showed a total red count of 4,700,000 and a white count of 10,000. Differential normal. Wassermann negative. Examination of the *spinal fluid* revealed no abnormality.

The patient remained in practically the same condition for about two weeks, vomiting at frequent intervals throughout this time. Vomiting gradually became less severe until the attacks ceased entirely. Nausea without vomiting then ensued for several weeks but gradually disappeared. The patient is still rather weak and emaciated, but is now able to eat freely of almost any food and is bright and cheerful mentally.

In view of the fact that a case had been observed previously, which was very similar to this one clinically, and that no lesions had been found at autopsy which could account for the symptoms, although the disease was so severe as to cause death, we determined to investigate the metabolism of this case as thoroughly as possible with the methods at our command. Inasmuch as Williams[1] has shown that in the pernicious vomiting of pregnancy a definite increase of the ammonia percentage in the urine is noted, as compared with the total nitro-

gen output, we were particularly interested in the urea-ammonia quotients of the blood and urine in this case.

The following tests for blood and urine urea were carried out by means of the Van Slyke[2] method. Ammonia was tested for by means of the Folin aeration method. The CO_2 content of the alveolar air was determined by means of the Fridericia method.[3, 4]

The following results were obtained:

Blood:	Urea 36 mg. per 100 c.c.	(October 26)
	" 34 " " " "	(November 3)
	" 39 " " " "	(November 22)
	" 36 " " " "	(December 3)
	" 35 " " " "	(December 14)
	Ammonia 23 mg. per 100 c.c.	(October 26)
	" 17 " " " "	(November 3)
	" 12 " " " "	(November 22)
	" 8 " " " "	(December 3)
	" 0 " " " "	(December 14)
Urine:	Urea 13.6 gm. output in 24 hours	(October 28)
	" 13.2 " " " " "	(November 3)
	" 12.8 " " " " "	(December 3)
	Ammonia 0.8 gm. in 24 hours	(October 28)
	" 0.5 " " " "	(November 3)
	" 0.38 " " " "	(December 3)
Alveolar Air: (CO_2 Tension)	October 26, 45 mm. of Hg.	
	November 3, 43 mm. of Hg.*	
	November 26, 46 mm. of Hg.	

An analysis of the above examinations reveals a very marked increase in the ammonia percentage of the blood. While, according to Folin and Denis[5] as well as all others who have worked upon this subject, only slight traces should be found in the blood normally—such slight traces, in fact, as to be scarcely perceptible by means of the ordinary quantitative test; in this case almost as much of ammonia as of urea was present in the blood. The urea percentage of the blood was found, however, to be only slightly above the limits of normal. The urine showed a slightly diminished urea output with a slightly increased ammonia excretion.* Examination of the CO_2 content of the alveolar air revealed absolutely no suggestion of acidosis, nor did the examination of the urine for acetone bodies reveal any sign of their increase at any time. Apparently, therefore, acidosis could be eliminated as a cause, *per se*, of the blood ammonia. Since it is in the liver that much, if not all, of the ammonia caused by protein metabolism is believed to be changed into the nontoxic urea,[6, 7, 8] the question suggests itself to us as to the possibility of a functional derangement of that organ being a factor in accounting for the symptoms and metabolic abnormalities found in this case.

Feeling that it would be desirable to apply the same methods which we had used in studying this case, to other individuals, both normal and abnormal, we have carried out similar studies routinely upon twenty different individuals. An analysis of this list reveals several pathological conditions associated with

*While these findings according to Ambard's law indicated a slight deficiency of the kidneys, this deficiency was certainly not sufficient for the symptoms noted in this case.

an increase of blood ammonia; this increase being found particularly in individuals suffering from failure of cardiac compensation. Acidosis has, however, been present at the same time in all of these cases. None of our normal cases (seven) have shown any demonstrable quantities of ammonia in the blood by the Folin method.

While we do not feel, from this limited study, that any sweeping conclusions can be drawn as to the origin and significance of an increased ammonia content of the blood, we feel that at least three facts have been demonstrated:

First: Increased amounts of ammonia appear in the blood in certain pathological conditions independent of any condition of acidosis, and are presumably due to functional disturbances of the liver.

Second: In the estimation of blood urea as a diagnostic procedure, simultaneous estimations of the blood ammonia should be carried out, since, otherwise, a considerable error in the urea readings may be produced in certain pathological conditions.

TABLE I

ALGER CO.

	DISEASE	UREA (GM.) PER 100 C.C. OF BLOOD	AMMONIA (GM.) PER 100 C.C. OF BLOOD	TOTAL OUTPUT OF UREA IN URINE IN 24 HOURS IN GM.	TOTAL OUTPUT OF AMMONIA IN URINE IN 24 HOURS IN GM.	CO_2 TENSION OF ALVEOLAR AIR	ACETONE BODIES IN URINE	REMARKS
1	Normal	0.02	None	.....	.....	46	.....	.....
2	Normal	0.027	None	.....	.....	42	.....	.....
3	Normal	0.018	None	.....	.....	40	.....	.....
4	Normal	0.02	None	.....	.....	39	.....	.....
5	Normal	0.038	None	.....	.....	41	.....	Arteriosclerosis.
6	Normal	0.022	None	.....	.....	40	.....	.....
7	Normal	0.014	None	.....	.....	43	.....	.....
8	Nephritis	0.068	0.003	7.2	0.6	26	None	Dyspnea.
9	Nephritis	0.095	None	4.8	0.3	40	None	No dyspnea.
10	Uremia	0.12	0.006	2.3	1.3	20	Acetone and diacetic	Cheyne-Stokes respiration.
11	Nephritis	0.068	None	3.	0.5	22	None	Dyspnea marked.
12	Myocarditis Nephritis	0.13	None	4.	0.8	29	None	Dyspnea marked.
13	Nephritis with Uremia.	0.131	None	.....	.....	27	None	Moderate dyspnea
14	Myocarditis?	0.031	0.017	.....	.....	28	Acetone and diacetic	Pulsating Liver
15	Myocarditis?	0.021	0.0015	8.5	1.3	22	Acetone	Dyspnea.
16	Myocarditis?	0.014	0.006	3.3	0.5	32	None	Chronic Passive Congestion of Liver
17	Alcoholic Cirrhosis of liver	0.036	0.01	12.2	0.49	40	None	No dyspnea.
18	Carcinoma head of pancreas. Marked jaundice	0.012	None	.....	.....	46	.....	No dyspnea.
19	Catarrhal jaundice	0.015	None	.....	.....	39	.....	No dyspnea.
20	Syphilis of liver	0.024	None	.....	.....	43	.....	No dyspnea.

Third: A case is reported characterized by nausea, vomiting and mental abnormalities (irritability, depression) in whose blood a large amount of ammonia was constantly found in spite of an absence of acidosis. This phenomenon we believe to have been due to a functional insufficiency of the liver.

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LABORATORY METHODS

THE USE OF FEHLING'S SOLUTION IN THE DETERMINATION OF BLOOD SUGAR*

By Hugh McGuigan, Ph.D., M.D., Chicago.

UP to the present time no agent has been so much used in blood sugar determinations as Fehling's solution. If one examines the Fehling solutions made up by the various formulas recommended, a great variation in the alkalinity is found. For example, the United States Pharmacopeia VII used 125 grams of KOH in 500 c.c.; United States Pharmacopeia VIII reduced this to 75 grams; and the present United States Pharmacopeia IX reduces this to 50 grams. This makes the alkalinity of the mixed fluid vary from 12.5% to 5%KOH. The U. S. Department of Agriculture Bureau of Chemistry, 1910, No. 107, revised, used 125 grams to 500 c.c.; Mathews' Physiological Chemistry uses the same strength; Fehling's original solution, final mixture was about 5.6% which he afterwards reduced to about 4.75% NaOH. Claude Bernard first used about 11.5% and later about 6% NaOH of the mixed fluids. Many other examples might be cited. On the whole, however, the final mixed fluid in common use varies from 5% to 12.5% KOH, and these limits may be extended.

If one examines the percentage of sugar reported in the literature purporting to be the normal level of the blood sugar, it will be found ranging all the way from about .03% or .05% to 0.150% for dogs.

In recent work in which was used both Benedict's method and the Bertrand method the author and his coworkers were struck by the differences in the yield. When for instance the Bertrand method gave .02% the Benedict method gave 0.1%. The explanation for this difference was found, and it is believed, also for the variations in the normal sugar concentration of the blood reported in the literature.

Our recent method of preparing the blood for the Bertrand titration was to remove the protein with picric acid. The picric does not interfere with Fehling's solution, and need not be removed until the precipitated cuprous oxid is filtered off, when the picric can be washed out readily. In precipitating the blood we used 10 c.c. of blood, laked, and made this to 150 c.c. with saturated picric solution. This was then filtered and an aliquot part used. We used 100 c.c. of the filtrate = 6.66 c.c. of blood.

The Fehling solution most used was the Allihn modification of Fehling solution, except that NaOH was used for KOH, and the solution was diluted with an equal quantity of water, as used by Morris.†

*From the Department of Pharmacology, Northwestern University Medical School.
†Morris: Clinical Laboratory Methods, p. 39.

The following is the exact formula:

I.	Copper sulphate	34.65 gm.
	Water to	1000. c.c.
II.	Rochelle salt	173.0 gm.
	Sodium hydrate	125.0 gm.
	Water to	1000. c.c.

When these are mixed, the NaOH concentration is 6.25% which is approximately that recommended by Hammersten, Remsen, and others. Sixty c.c. of this mixed fluid and 100 c.c. of the picric filtrate were heated to boiling and then the Fehling poured into the picric solution, and the mixture kept boiling for two minutes. With these volumes there is no bumping, and the changes due to evaporation are reduced to a negligible quantity. This solution used on known dextrose solutions *in water* will give good results so it is considered satisfactory in blood sugar work.

In seeking for the reason of the great difference between this method and the Benedict colorimetric method, we naturally suspected a sugar of the maltose type and so hydrolysed, as we thought, and obtained theoretical results. There were many facts, however, especially the short time necessary to complete the "hydrolysis" which made us doubt this explanation, and we finally found that the addition of the acid directly to the Fehling solution, i.e., lessened alkalinity, gave the same result. This striking fact was fully confirmed by the use of a Fehling solution in which the alkali strength approximates that given in the present United States Pharmacopeia which is the approximate strength used by most investigators who have reported the higher levels of the normal blood sugar.

There is a striking difference between a blood filtrate and a water solution of sugar. In a water solution of 0.1% dextrose we have never found more than 10% to 15% variation no matter what the alkali concentration of the Fehling solution used. In blood filtrates, however, the strong alkalies in Fehling prevent the precipitation of the copper.

Similarly dextrose added by *glycolysed* blood or to yeast fermented sugar filtrates cannot be recovered completely when strongly alkaline Fehling's solutions are used, but can be recovered completely when the final strength is about the equivalent of 5% KOH, as recommended in the present Pharmacopeia. Increasing the copper has much the same influence as decreasing the alkalinity. It is apparent, therefore, that the relation of the copper to the alkali is important. It is usually only the alkali, however, that is changed.

An example of the differences due to the variations in the alkalinity of Fehling's solution will suffice: A dog was bled from the jugular, no anesthesia, the blood was oxalated and precipitated as above:

I.	1. Lewis-Benedict method gave	0.106%
	2. Fehling 6.25% NaOH in the mixed solution gave	0.018%
	3. Fehling 2.5% NaOH in the mixed solution gave	0.090%
	4. Fehling 2.0% NaOH in the mixed solution gave	0.105%

The dilutions here were made by adding Rochelle salt solution to Solution II above. In this way the alkali only varied.

II. Dog bled in same way:
In this solution the copper is about double that in I.
1. KOH in Fehling's 10% $CuSO_4$ 7% 0.09%
2. KOH " " 5% " 7% 0.157%

III. In water solutions, the copper concentration remaining the same:
5% KOH = 0.115
12.5% KOH = .106
or a variation of relatively slight importance.

This variation in the alkalinity of Fehling's solution seems to be the main cause of the great differences in the literature regarding the level of the normal blood sugar. The error is due to the assumption made by all writers; viz., that the sugar in a protein-free blood filtrate will act toward Fehling's solution as a solution of dextrose in water. Such an assumption is apparently untenable. Why a weaker alkaline Fehling should give a greater yield than a strong one is hard to explain. It seems to be because such a solution is more sensitive. It is not because the stronger alkali destroys the sugar, for two reasons: first, in a water solution of dextrose the difference between the stronger and weaker alkaline Fehling is relatively slight; second, if blood filtrate and stronger Fehling be boiled for two minutes and then the alkalinity be reduced by the addition of acetic acids, the yield of the weaker alkaline Fehling can be obtained. These results obtain no matter what agent is used to remove the proteins. Alcohol, sodium sulphate, colloidal iron, and picric acid all give a higher blood sugar yield when a 5% KOH Fehling is used than when 12.5% KOH is used. The weaker solution gives results which agree more closely with Benedict's colorimetric method, and is, I think, much more accurate than that obtained from the more strongly alkaline fluid.

THE PRESERVATION OF ERYTHROCYTES OF A KNOWN GROUP FOR ISOAGGLUTININ GROUP DETERMINATION*

By M. G. Wohl, Omaha, Nebr.

THE most efficient method for testing donors and recipients for blood transfusion is that of Moss as modified by Minot. A complete and accurate account of the technic employed has been given recently by Brem.

I have found this procedure very simple and practicable. However, a great difficulty that one encounters is the necessity of having on hand serum and red blood corpuscles of a known group, two or three. The serum, if kept sterile, will retain its agglutinative power for a month or longer. The suspension of red blood corpuscles, however, will not keep longer than forty-eight hours. The preservation of the red blood corpuscles then is a desideratum of great importance. With this aim in view the various methods devised for preservation of sheep cells for the Wassermann reaction have been applied to citrated suspension of human red blood corpuscles of known groups, two and three. The ad-

*From the Pathological Department, Nicholas Senn Hospital.

dition of formaldehyde to sodium citrate solution has given us very satisfactory results. The formalized cells preserved as long as four weeks showed neither hemolysis nor lost the property of becoming agglutinated with the serum of the proper group. The serum of the known group was preserved in sterile Wright's blood capsules.

The stock solution is made up by adding 0.5 c.c. of 40 per cent formaldehyde solution to 500 c.c. of 0.85 per cent salt solution containing 2 per cent sodium citrate. To 1 c.c. of this solution is added three drops of blood and the suspension is preserved in cotton stoppered test tubes. When needed, the corpuscles are brought into suspension by gently shaking the test tube.

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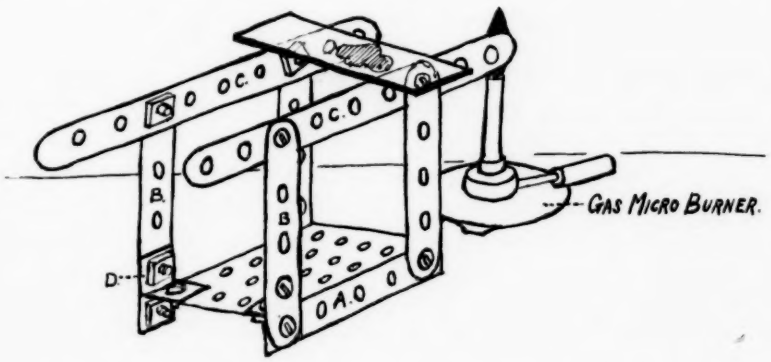
CONSTRUCTION OF APPARATUS USING THE STRUCTURAL STEEL TOY

By Simon B. Kleiner, M.D., New Haven, Conn.

THE piece of apparatus illustrated is for holding slides while they are being stained for microscopic examination. It is of practically the same design as those found in many pathological and clinical laboratories, but it has been constructed from the most inexpensive outfit of miniature structural steel, the entire cost of the apparatus being only ten cents.



The mode of construction of the apparatus is self-evident. It consists essentially of a base (A) with four uprights (B) which support two parallel bars (C). Small elbows (D) hold two of the uprights rigid. If desired, the uprights may be bent slightly to approximate the parallel bars to within a space of about one and a quarter inches of each other.

The slides to be stained are placed upon the parallel bars and can then be stained and heated. The apparatus is small enough to be placed in a pan to catch the stain and washings.

The author does not claim any originality for this apparatus, but wishes rather to suggest the use of the structural steel toy to construct laboratory apparatus, surgical splints, and orthopedic appliances. This toy can be purchased in any city, at a very moderate price, and working models of apparatus, or apparatus itself, of neat design, can be easily constructed with it.

Moreover, any design found unpractical can be taken down quickly, and an entirely different model can be as readily built again.

CLARIFICATION OF COMPLEMENT IN RELATION TO ITS PRESERVATION*

By Norman E. Williamson, M.D., Stockton, Calif.

THE usual directions for the preparation of complement include the pipetting of the clear (?) serum after its separation. Such a serum will of course contain millions of cells. It is well known that human serum may contain amboceptors for the guinea pig cells. Guinea pig complement would not be particularly active against guinea pig cells. Nevertheless, in order to prevent the possibility of a false positive from such an action, I am in the habit of centrifugating my complement to perfect clearness, that is from twenty minutes to half an hour.

I find that complement so clarified will preserve its properties with slight gradual loss for days in the ice box without freezing. It will lose about one-third of its strength in five days. I arbitrarily discard complement which is weaker than two-thirds of the strength of average complement, that is 0.02 c.c. for a unit in the Noguchi system. Thus, if the material proves to be stronger than average on titration, it is still useful for many days. Titration at time of use is, of course, necessary.

*From the State Hospital, Stockton, Calif.

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EDITORIALS

Resolutions Passed by the Faculty of the University of Michigan Medical School, April 2, 1917

THE Faculty of the University of Michigan Medical School assembled on the above mentioned date and passed the following resolutions:

1. It is the opinion of the Faculty of the University of Michigan Medical School that in meeting the demands for medical officers in the National service, the military authorities should give first preference for enlistment to the members of the medical classes of the past two years, viz.: 1915 and 1916.

Note.—These young men have recently finished their medical courses and having taken in part or altogether their hospital training, should have the latest and best information in scientific medicine, and not having as yet established themselves in practice, are best fitted to be selected for military service.

2. In view of the probably urgent demands for trained medical men, the Faculty of the University of Michigan Medical School desires to place itself on record as being ready and willing to make its courses of instruction continuous through the summers of 1917 and 1918. This proposition will be submitted to the various State Boards of Licensure for their approval.

Note.—If this provision goes into effect, a week after the close of the present session, the session of 1917-18 will begin. Those who are now Juniors will become Seniors and may be graduated in January, 1918.

Note.—In taking this step, not only the military demands upon the medical profession, but civil demands as well are taken into consideration.

3. Taking into consideration the future needs of the country for trained medical men, it is the opinion of the Faculty of the University of Michigan Medical School that it is advisable for the undergraduate medical students to complete their course of instruction and not to enlist.

4. The Faculty of the University of Michigan Medical School recommends that not less than two hours per week be set aside for the military drill of undergraduate students, and that in addition to the ordinary infantry drill, we recommend training along the lines developed by the Clinical Society of Albany, and known as the "Albany Plan."

Note.—The medical officer should first of all be a soldier. This is necessary in order to make him most efficient as a medical officer.

5. That copies of these resolutions be furnished for suggestions of approval or disapproval to the following bodies:

(1) The Surgeons General of the Army and Navy.
(2) The National Medical Committee on Preparedness.
(3) The National Research Council.
(4) The Faculties of other medical schools.

6. That a list of the graduates of the classes of 1915 and 1916, with their standing while in the school and their present addresses, be sent immediately to the Surgeons General of the Army and Navy.

In compliance with the above resolutions I am hereby submitting them to you, and will be grateful for immediate expressions of opinion concerning them.

I have the honor to be, yours most respectfully,

VICTOR C. VAUGHAN, DEAN,
University of Michigan Medical School.

The Toxemias of Pregnancy

THE strain to which the metabolic functions of the maternal organism is subjected in pregnancy is frequently so great that toxic substances accumulate to such an extent as to produce symptoms not infrequently ending in death. In the earlier months these manifest themselves on the digestive functions, producing the vomiting of pregnancy, a symptom that is so common as to be considered as a more or less normal sign of the condition. Sometimes, however, the toxemia even at this stage is so severe that the vomiting becomes intractable—the so-called pernicious vomiting. Later in pregnancy the maternal organism, as a rule, develops some means of defense against the toxemia, and the digestive symptoms disappear; but sometimes this defensive mechanism seems suddenly to break down so that the toxemic symptoms again assert themselves, affecting now, however, not so much the digestive apparatus as the nerve centers, and thus producing

eclamptic convulsions. The defensive mechanism against the development of these later symptoms seems to break down often with great suddenness. A case that is apparently running a perfectly normal course may unexpectedly develop eclampsia, although usually the accumulation of the toxic substances in the mother manifests itself by the gradual rise in diastolic pressure and often by the presence of albumin in the urine. Such a picture of the functional pathology of the toxemias of pregnancy may not at first seem to fit in with all the observed facts, but it does so closely enough to make it serve as a useful hypothesis from which to attack this most difficult problem.

The first question that presents itself concerns the nature of the toxic substance. According to the above hypothesis, this toxin acts over a long period of time; its action, in other words, is a cumulative one, so that it is not surprising to find that only negative results are obtained when an attempt is made to cause the symptoms to develop in a normal animal into which the blood of an eclamptic patient is injected. If positive results could be obtained in such an experiment, it would be rational to attempt the isolation of the toxic substance from the maternal blood, but since this is not the case, the only procedure open to us lies in patiently searching for possible metabolic abnormalities in the maternal organism. Three of these have recently received a considerable degree of attention; namely, a faulty metabolism of the amino acids; an improper relationship between ammonia and urea; and an increase in the H-ion concentration of the blood —in other words, a condition of acidosis. Losee and Van Slyke have recently contributed a most important article bearing on these possibilities. With regard to amino acid metabolism, they found, in twenty-three cases, that the amino nitrogen of the urine did not exceed the amount present in normal individuals (i. e., 3.6 per cent of the total N) except in three of the cases, but even in these the increase was not beyond that found in normal pregnancy, where there is a tendency for the amino nitrogen to be a little above the physiological level.

Since it is possible that a failure in amino acid metabolism might exist without going so far as to appreciably affect the amino acid content of the urine, the blood was also examined for an accumulation of these bodies, and again with negative result. "Not a single one of the ten eclamptic women whose blood was examined for amino nitrogen showed a figure outside the range of 4-8 mg. per 100 c.c. noted . . . . . in a series of normal men." Quite clearly, then, faulty metabolism of amino acids is not responsible for the toxemia of pregnancy, and the question naturally arises as to whether the excretion of the end products of protein metabolism, namely, ammonia and urea, might not be abnormal. The urea of the blood and urine and the ammonia of the urine were therefore determined, and with significant results. Thus, the urea of the urine, in relationship to the total nitrogen, was usually below the average normal; indeed, except in cases in which the examination was made one or more days *post partum*, it was below 70 per cent of the total nitrogen, values as low as about 50 per cent being obtained in a considerable number of the cases. The relative amount of ammonia was also higher than normal, although not so strikingly so as that of the urea. Very high ammonia figures were, however, obtained in six cases of pernicious vomiting, namely, always above 16.9 and sometimes as high as 30, the figures for normal men being usually given as varying between 3 and 11 per cent,

according to diet. The relatively high ammonia excretion may in part be due to the influence of starvation which in itself is well known to raise the ammonia ratio; but even admitting this, there can be no doubt that considerable diagnostic value attaches to an observation of the ammonia. It is pointed out that the relatively low urea and high ammonia is suggestive of the condition found by Nencki and Pavlov in the urine of dogs in which the liver had been partly removed from the circulation by the establishment of the Eck fistula. It is of further significance that such animals often suddenly develop symptoms which have some similarity to those of eclampsia, especially when the animals are fed exclusively on meat.

To investigate the possibility that acidosis might be responsible for the symptoms, Losee and Van Slyke examined the carbon dioxide combining power of the plasma by the method already described by them.[1] Sufficient results have now been collected to permit us to state that the blood of normal persons does not take up less than 55 c.c. nor more than 75 c.c. CO_2 per 100 c.c. of plasma; still narrower normal limits, but at a somewhat higher level, being possible when the method described by Austin and Jonas[2] is employed. The general average may be taken as 65 c.c. CO_2 per 100 c.c. plasma. Using the CO_2 tension of alveolar air as an indicator Hasselbalch and Gammeltoft have shown that a slight degree of acidosis exists in normal pregnancy. Values below the normal minimum (55) were observed by Losee and Van Slyke on at least one day in ten of fourteen cases of pregnancy examined. In cases of toxemia, whether of the eclamptic or vomiting type, no greater a degree of acidosis than that present in normal pregnancy was observed to occur. The CO_2 combining power of the plasma never approached anywhere near the low values of 30 c.c. CO_2 per 100 c.c. of plasma found to be present in cases of typical acidosis as met with in diabetes and nephritis.

The great importance of this investigation lies in the perfectly definite and final evidence which it supplies that neither faulty metabolism of the amino acids nor a condition of acidosis is accountable for the symptoms of the toxemia of pregnancy. The results indicate, however, that something is radically wrong with that stage in the metabolism of nitrogen which is responsible for the final balance between the urea and ammonia excretions. One other very important outcome of the investigation goes to show that there is not necessarily any relationship between the ammonia excretion and the degree of acidosis. Thus, in the cases of pernicious vomiting where the NH_3 excretion was abnormally high, the plasma did not absorb any less CO_2 than that found in cases of normal pregnancy.

[1]Losee, J. R., and Van Slyke, D. D.: The Toxemias of Pregnancy, Am. Jour. Med. Sc., 1917, cliii, 94.

[2]The modification referred to consists in drawing the blood directly from the patient's vein through tubing, which passes to the bottom of a centrifuge tube containing some oxalate crystals and paraffin oil. The latter floats on the surface of the blood and thus prevents loss of CO_2 during the centrifuging. The experimental error can be still further cut down by saturating the oxalated blood with CO_2 at 6 per cent of an atmosphere. The rationale for these precautions is that the CO_2 combining power of plasma is influenced by the CO_2 tension of the blood existing at the time when the corpuscles and plasma are separated. Austin, J. H., and Jonas, L.: Clinical Studies in Acidosis, Am. Jour. Med. Sc., 1917, cliii, 81.

—*J. J. R. M.*

Hodgkin's Disease

FOR about eight years Bunting and Yates have been engaged in an intensive study of Hodgkin's disease, which they believe is an infectious disease caused by an organism, B. hodgkini,[1, 2, 3] a pleomorphic diphtheroid, which stains by Gram's method. Beside being an infectious disease, the complex has also the destructive potentialities of the malignant neoplasm, and these two facts have been fundamental in the method of treatment which these two workers have perfected.

For the purpose of comparing the effects of treatment in different cases Bunting and Yates[4] have grouped them, using a classification which is an amplification of Trousseau's earlier one. Trousseau classified Hodgkin's disease into latent, progressive, and cachetic cases. Bunting and Yates' modification is as follows (the schema indicates the number of cases they report):

All cases (63)	Acute cases (5)			Group 1	5 cases
	Chronic cases (58)	Stage 1 (8)	Early	Group 2	5 "
			Late	Group 3	3 "
		Stage 2 (25)	Early	Group 4	11 "
			Late	Group 5	14 "
		Stage 3 (25)	Early	Group 6	14 "
			Late	Group 7	11 "

In the acute cases the blood picture,[5] the most reliable single diagnostic evidence, is often obscured by the leucocytosis. "It is fortunate," say the authors, "that the incidence of this form of the disease is comparatively slight, for it is about as amenable to treatment as is the mastitis carcinomatosa of lactation or melanoma."

Group 2, composed of *incipient cases*, has a blood picture that is characteristic, and this picture appears early. In these cases the portal of entry has been recently established and only a few adjacent glands are beginning to be palpable. The only difficulty is that the diagnosis at this stage is fortuitous and depends largely upon careful physical examination, routine blood counting, and accurate interpretation of tuberculin tests.

Group 3, in which the *early cases* are placed, has the characteristic blood picture. In these there is unmistakable involvement of a primary group of glands but without demonstrable extension. In this group and every other more advanced one the occurrence of a specific bacteriemia of some extent is virtually certain at one or more of the positive phases.

Group 4. In this group are the *moderately advanced cases* in which the blood picture is positive but not of an early type. In them more than one of the main superficial gland groups are involved but extension to the mediastinal or peritoneal glands can not be more than suspected.

Group 5. In these, the *advanced cases*, the blood picture may be like

[1]Bunting and Yates: Jour. Am. Med. Assn., 1913, lxi, 1803; Ibid., 1914, lxii, 516.
[2]Bunting, Yates, and Kristjanson: Ibid., 1914, lxiii, 2225.
[3]Bunting and Yates: Arch. Int. Med., Aug., 1913.
[4]Bunting and Yates: Jour. Am. Med. Assn., 1917, lxviii, 747.
[5]Bunting: Bull. Johns Hopkins Hosp., 1911, xxii, 248.

that of Group 4, or it may show a reduction in nearly all the cell forms. In them there is always demonstrable involvement of deep structures.

Group 6 is composed of *cases which are probably hopeless, but capable of palliation.* The blood picture is of the late type.

Group 7 is made up of *cases with lethal involvement and probably beyond palliation.*

The treatment used by Bunting and Yates is based upon the belief that the disease is a chronic infection of lymphoid tissue, and that there is evidence that the affected persons are incapable of a permanent protective reaction without assistance. They therefore attempt to assist the patient by thorough excision of as much infected tissue as possible; to remove or heal possible portals of entry (tonsils, teeth, dermatitides, bronchitides, etc.); to add to the patient's resistance by the use of immune sera; and to reduce or remove as much potential or existing lymphoid tissue as may be possible by the use of the x-ray.

The results attained are of exceeding interest when taken in conjunction with the previous mortality expectancy figure of 100 per cent.

Group	1.	Estimated	possibility	of	recovery,	less than	5%
"	2.	"	"	"	"	from 80 to	90%
"	3.	"	"	"	"	from 60 "	70%
"	4.	"	"	"	"	from 30 "	40%
"	5.	"	"	"	"	from 5 "	10%
"	6.	"	mortality				100%
"	7.	"	"				100%

These figures mean that in its early stages Hodgkin's disease is a very curable disease, in which respect it is quite like carcinoma. In each case early recognition demands patient care.

—*P. G. W.*